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CHLAMYDIA TRACHOMATIS INFECTION IN SWEDEN: TIME TRENDS, RISK FACTORS, AND PREVALENCE

Inga Veličko

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CHLAMYDIA TRACHOMATIS INFECTION IN SWEDEN: TIME TRENDS, RISK FACTORS, AND PREVALENCE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Inga Veličko

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Principal Supervisor:
Professor Pär Sparén
Karolinska Institute
Department of Medical Epidemiology and Biostatistics

Co-supervisor(s):
PhD Sharon Kühlmann-Berenzon
Public Health Agency of Sweden
Department of Public Health Analysis and Data Management

Associate Professor, MD Lena Marions
Karolinska Institute
Department of Clinical Science and Education

Associate Professor, Björn Herrmann
Uppsala University
Department of Medical Sciences,
Section of Clinical Bacteriology

Opponent:
PhD Elizabeth Torrone
Centers for Disease Control and Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD & TB Prevention
Surveillance and Special Studies Team

Examination Board:
Associated Professor Nicola Orsini
Karolinska Institute
Department of Global Public Health

Associate Professor, MD Petra Tunbäck
University of Gothenburg
Department of Dermatology and Venereology

Professor, MD Anna Mia Ekström
Karolinska Institute
Department of Global Public Health

In the memory of my beloved mother, Lyubov Veličko

Уходят наши матери от нас,
уходят потихонечку,
на цыпочках,
а мы спокойно спим,
едой насытившись,
не замечая этот страшный час.
Уходят матери от нас не сразу,
нет —
нам это только кажется, что сразу.
Они уходят медленно и странно
шагами маленькими по ступеням лет.
…
Все удаляются они,
все удаляются.
К ним тянемся,
очнувшись ото сна,
но руки вдруг о воздух ударяются —
в нем выросла стеклянная стена!
Мы опоздали.
Пробил страшный час.
Глядим мы со слезами потаенными,
как тихими суровыми колоннами
уходят наши матери от нас…

Евгений Евтушенко, 1960 г.

Our mothers depart from us,
gently depart
On tiptoe,
but we sleep soundly,
stuffed with food,
and fail to notice this dread hour.
Our mothers do not leave us suddenly,
no —
it only seems so ‘sudden’.
Slowly they depart, and strangely,
with short steps down the stairs of years.
…
They withdraw ever further,
withdraw even further.
Roused from sleep,
we stretch toward them,
but our hands suddenly beat the air —
a wall of glass has grown up there!
We were too late.
The dread hour had struck,
Suppressing tears, we watch our mothers,
in columns quiet and austere,
departing from us.

By Yevgeny Yevtushenko, 1960. Translated by Albert Todd and James Ragan.

POPULAR SCIENCE SUMMARY OF THE THESIS

Chlamydia infection is one of the most common bacterial infections transmitted via sexual contact. Every year 127 million people are infected with chlamydia in the world. Chlamydia is very common among adolescents and young adults. What is also striking is that the infection often has no symptoms and infected people could be unaware of it. If an infection is untreated, it can lead to serious complications in the organs involved in sexual reproduction, causing chronic pain and possible difficulties to conceive later in life. Researchers have identified several factors with an increased risk to be infected with chlamydia infection, such as younger age, sexual contact with casual partners without using condoms. Therefore testing for chlamydia after having unprotected sex is important, even if one does not have symptoms. Testing, treatment, and identification of sexual partners are important components to control the spread of chlamydia in the population.

It is essential to follow the trends of chlamydia in order to monitor the changes, interpret them, and adjust control measures, if necessary. Taking into consideration testing intensity in the population allows a better understanding of the number of diagnosed chlamydia cases. Testing, which is widely accessible in Sweden, as well as following treatment and sexual partner identification, can affect chlamydia presence in the population. Therefore, knowledge on chlamydia occurrence in the population is important for planning control measures and possible prevention campaigns.

In this thesis, we focus on a better understanding of behaviours associated with chlamydia and repeated testing, as well as the distribution of infection over time and in the population. In study I, we found that high-risk behaviours (e.g., more than 6 sexual partners, alcohol use before a sexual encounter, being young) are associated with chlamydia infections. In study II, individuals characterised of several high-risk behaviours were tested more often repeatedly for chlamydia, suggesting that they have followed public health messages to be tested if they consider themselves at risk for chlamydia. In study III, we concluded that chlamydia cases reported during the past 10 years were more likely to mirror the real situation with chlamydia in the population since we capture in our surveillance system only a part of infections without symptoms. Moreover, testing volumes play an important role in interpreting trends of chlamydia infection. Additionally, in study IV, for the first time in Sweden, we estimated the occurrence level of chlamydia infection in the population of 15-29 year olds, and it was comparable to other high-income countries.

In summary, we gained a better understanding of risk factors for chlamydia infection and repeated testing, chlamydia trends over time, and distribution in the population, which will help to assess and plan future control measures in Sweden.

ABSTRACT

Chlamydia trachomatis (chlamydia) infection is the most common bacterial sexually transmitted infection (STI), with an estimated 127 million new cases occurring every year worldwide. Due to the asymptomatic nature of the infection, individuals may carry it for a long time and transmit without knowing about it. Untreated chlamydia may lead to serious sequelae of the reproductive tract, causing pelvic inflammatory disease, chronic pain, ectopic pregnancy, and tubal factor infertility. Thus, early detection of infected individuals via testing (opportunistic testing), treatment, and partner notification may prevent further transmission of infection.

In this thesis, we aimed to gain an extended understanding of chlamydia epidemiology at an individual and population-based level. The thesis is based on the data from a cohort study in the urban STI clinic, as well as chlamydia cases and tests reported to the national infectious diseases register SmiNet-2 at the Public Health Agency of Sweden. We employed various methodologies to answer our study questions. In Studies I and II, we used data from a cohort study, which we analysed cross-sectionally. We found that being 20-24 years old, having 6 or more sexual partners during the previous 12 months, using alcohol before sex, reporting all type of sexual activities during the last sexual contact, and testing due to partner notification were independently statistically significantly associated with increased risk to test positive for chlamydia. Furthermore, we identified four groups (latent classes) of behaviour patterns among men and three among women in our cohort. The classes characterized by high-risk sexual behaviour were associated with statistically significantly increased 2-fold odds for lifetime repeated testing for chlamydia among men and women. Women in the high-risk behaviour class had also 2-fold increased odds to test repeatedly during the previous 12 months. This indicates that individuals at higher risk for chlamydia acquisition had adhered to the public health messages to test if at risk for infection. In Study III, we used time series analysis to explore how chlamydia trends changed over time, by comparing two periods: before and after the discovery of a new variant of *Chlamydia trachomatis* in Sweden. We analysed data nationally and by two types of counties, grouped according to their ability to identify new variant at the time of discovery. We also adjusted chlamydia trends to the testing intensity. We found that chlamydia trends were increasing since the mid-1990s up to 2004, as was testing, suggesting that chlamydia notification trends were driven by the testing. On the other hand, during 2009-2018 chlamydia trends were decreasing, despite increasing testing intensity, suggesting that chlamydia cases were not driven by testing, and most probably, these trends reflect true chlamydia incidence rates in the population. In Study IV, we continued to explore the reason for the decreasing chlamydia trends during 2009-2018 by estimating chlamydia prevalence via mathematical modelling. Indeed, we estimated a decrease in chlamydia prevalence among 15-29 year old men and women during this period, which supports our hypothesis.

In conclusion, the risk factors independently associated with chlamydia diagnosis are in line with previous studies. Furthermore, our results suggest that individuals in the high-risk

sexual behaviour latent classes are more likely to test repeatedly for chlamydia, suggesting absorption of public health messages. Moreover, chlamydia trends were not driven by testing intensity during 2009-2018, suggesting a true decrease in chlamydia incidence rates in the population. By estimating the decrease in chlamydia prevalence during this period, we were able to support the hypothesis on the reason for observed declining chlamydia trends. Prevention work should be continued to reach asymptomatic individuals by testing and prevention messages. Further studies should investigate the role of other components of chlamydia control strategy on chlamydia trends to disentangle their input and plan for possible future alterations.

Keywords: chlamydia infection, epidemiology, risk factors, incidence rate, opportunistic testing, repeated testing, prevalence, mathematical modelling, time series analysis, latent class analysis, regression analysis

LIST OF SCIENTIFIC PAPERS

I. **Veličko I**, Ploner A, Sparén P, Marions L, Herrmann B, Kühlmann-Berenzon S. Sexual and testing behaviour associated with *Chlamydia trachomatis* infection: a cohort study in an STI clinic in Sweden. *BMJ Open*. 2016 Aug 26;6(8):e011312. doi: 10.1136/bmjopen-2016-011312.

II. **Veličko I**, Ploner A, Marions L, Sparén P, Herrmann B, Kühlmann-Berenzon S. Patterns of sexual behaviour associated with repeated chlamydia testing and repeated chlamydia infection in men and women at urban STI-clinic in Stockholm: a latent class analysis.
[Submitted manuscript]

III. **Veličko I**, Ploner A, Sparén P, Herrmann B, Marions L, Kühlmann-Berenzon S. Changes in the trend of sexually acquired chlamydia infections in Sweden and the role of testing: a time series analysis. *Sexually Transmitted Diseases*. October 27, 2020 - Volume Publish Ahead of Print - Issue - doi: 10.1097/OLQ.0000000000001318.

IV. **Veličko I**, Hansson D, Colombe S, Kühlmann-Berenzon S. Estimation of population-based *Chlamydia trachomatis* prevalence among 15-29 years old individuals in Sweden: a mathematical modelling study.
[Manuscript]

RELATED PUBLICATIONS

(Not included in the thesis)

Riera-Montes M, **Velicko I**. The Chlamydia surveillance system in Sweden delivers relevant and accurate data: results from the system evaluation, 1997-2008. *Euro Surveill*. 2011 Jul 7;16(27). pii: 19907. Erratum in: *Euro Surveill*. 2011;16(28). pii: 19919.

Decraene V, Kühlmann-Berenzon S, Andersson Franko M, **Veličko I**. Differences in travel-related incidence of chlamydia by age groups, gender and destination: Sweden 2000-2013. *Travel Med Infect Dis*. 2018 Sep - Oct; 25:42-49. doi: 10.1016/j.tmaid.2018.02.008.

CONTENT

LIST OF ABBREVIATIONS

AIC	Akaike information criterion
BIC	Bayesian information criterion
CI	Confidence interval
CrI	Credibility interval
EP	Ectopic pregnancy
EU/ EEA	European Union/ European Economic Area
IR	Incidence rate
IRR	Incidence rate ratio
LC /LCA	Latent class/ Latent class analysis
LRT	Likelihood ratio test
MSM	Men who have sex with men
NAAT	Nucleic acids amplification test
NGO	Non-governmental organization
nvCT	New variant (Swedish variant) of *Chlamydia trachomatis*
OR/ adjOR	Odds ratio/ Adjusted odds ratio
P	P-value
PID	Pelvic inflammatory disease
PHAS	Public Health Agency of Sweden
PIN	Personal identification number (*personnummer*)
POC	Point of care tests
RR/ adjRR	Risk ratio/ Adjusted risk ratio
SIR	Susceptible-Infected-Recovered
STI	Sexually transmitted infection
TSA	Time series analysis
TFI	Tubal factor infertility
UI	Uncertainty interval
WHO	World Health Organization

1 INTRODUCTION

Chlamydia trachomatis infection (chlamydia) is one of the most common curable bacterial sexually transmitted infections (STIs), with an estimated 131 million new cases worldwide among populations aged 15–49 years in 2012 (1). Chlamydia is an important public health problem not only because of its high case rates and because of the potential to cause reproductive sequelae in both men and women (2, 3) but also due to the broader aspect of being a part of the sexual health well-being. Therefore, countries are urged to work towards "ensuring universal access to sexual and reproductive health care services, provide family planning, information and education, and integrate reproductive health into national strategies and programmes" as stated by the United Nations (UN) Sustainable Development Goal (SDG) 3 sub-goal 3.7 (4). In Sweden, sexual well-being, where definition includes also the absence of STIs, is part of the national strategy for sexual and reproductive health and rights (SRHR) (5).

Sweden has a long tradition of epidemiological and microbiological surveillance of chlamydia infection. To control chlamydia, several measures are in place with the goal to identify and treat cases of chlamydia and prevent sequelae in the population. As part of my work with epidemiological surveillance at the Public Health Agency of Sweden, data analysis and interpretation is a daily task. However, sometimes interpretation is not straightforward and requires additional data. Hereby several hypotheses formed the basis for the studies in this thesis. This thesis aims to provide improved knowledge on chlamydia epidemiology in Sweden and adds evidence to a pool of chlamydia research.

2 BACKGROUND

2.1.1 Epidemiology

2.1.1.1 Chlamydia trachomatis pathogen and clinical manifestations

Chlamydia infection is caused by *Chlamydia trachomatis,* which is an obligate intracellular gram-negative bacterium with an incubation time between 1-3 weeks (6). *Chlamydia trachomatis* can cause a range of clinical manifestations, which are linked to 19 serovars and variants (7). Serovars A–C are responsible for hyperendemic blinding trachoma and are prevalent in tropic countries (6). Urogenital chlamydia caused by the serovars D–K can manifest as urethritis, cervicitis, and proctitis, characterized by pain and discharge, and is most common in heterosexual populations. The most common serovars found among men who have sex with men (MSM) are D, G, and J (8), while serovars E and D are more often reported in heterosexual populations (9, 10). Another less common clinical presentation of these serovars is neonatal conjunctivitis and neonatal pneumonia. Finally, serovars L1–L3 cause *Lymphogranuloma venereum*, which re-emerged in 2003 in the Netherlands and later elsewhere (11, 12), were exclusively reported in MSM (13). In this thesis we are focusing on the population, where the most of chlamydia transmission is ongoing, that is the heterosexual population (which is defined below).

Chlamydia infections are often asymptomatic: about 75% of women and 50% of men do not present any symptoms (6). In another study, it was estimated that 11% of chlamydia infections in men and 6% in women become symptomatic (14).

Apart most common urogenital presentation of chlamydia, the prevalence of extragenital chlamydia infection is not uncommon, especially in MSM. In a recent review, extragenital chlamydia in women a median of 8.7% was reported for rectal infection and a median of 1.7% for pharyngeal chlamydia (15). This study, which is based mostly on the review of the studies carried out in STI clinics, also reported that most of the chlamydia infections were asymptomatic in women (36–100% of rectal and 100% of pharyngeal chlamydia) (15). Among men who have sex with women, a median prevalence of 7.7% was reported for rectal and 1.6% for pharyngeal chlamydia infections (15). However, it is noteworthy that some women who test positive for rectal chlamydia deny having had anal sex. This has been suggested as possible due to the autoinoculation of the rectum from vaginal secretions (16, 17). Meanwhile, rectal chlamydia identified among men who have sex with women who did not report anal sex, could also be explained by inaccurate reporting of sexual contacts with other men (15) or by possible inoculation during sexual practices with women (e.g., cunnilingus) (18).

Several studies have also reported *Chlamydia trachomatis* co-infection with other sexually transmitted pathogens, such as human papillomavirus, *Mycoplasma genitalium*, and *Neisseria gonorrhoeae* (19-24), with prevalence depending on the setting, the country, and so called “key populations” (25). Studies have also reported that *Chlamydia trachomatis* was independently (26) in co-infection with human papillomavirus (20) associated with cervical

intraepithelial neoplasia. An important finding also reported in the literature is that the presence of *Chlamydia trachomatis* can facilitate transmission of HIV through the inflammation in the tissues (27-29).

2.1.1.2 Transmission routes

In this thesis, we are not describing the population based on their sexual identity, but their sexual orientation (30). Sexually acquired chlamydia can be transmitted via oral, vaginal, or anal sexual contacts and clinical presentations therefore reflect the sexual contact practices. Based on that and the biological sex of the person, possible routes of the transmission are categorised as follows: heterosexual transmission (sexual contacts between persons of opposite sex), homosexual transmission (sexual contact between persons of the same sex, specifically MSM) and bisexual transmission (sexual contact with persons of both sexes). Heterosexual transmission is the predominant route of transmission reported in the literature, with 86% transmitted heterosexually and 5% transmitted in MSM (31). Because chlamydia infection is reported elsewhere and in Sweden predominantly heterosexually transmitted (32), the focus in this thesis is on this population, with only some occasional remarks on other populations at risk for chlamydia. Chlamydia infection can be transmitted from mother to newborn child during vaginal delivery; however, this is a rare transmission route due to prenatal screening in some countries (33). While in Sweden no official recommendations exist, pregnant women are offered testing for chlamydia around week 10-12 of pregnancy, however the screening is done at varying degrees in the country (34).

Transmission probability for chlamydia varies depending on the type of sexual contact, number of sexual acts and partnership length. It has been estimated that transmission probability was 2.0% (interquartile range: 1.7–2.2%) per vaginal sex act and 5.8% (interquartile range: 3.0–8.3%) per anal sex act, both from male-to-female and female-to-male (17). Another study estimated transmission probability of 32.1% (95% credibility interval (CrI) 18.4–55.9%) from male to female and transmission probability of 21.4% (95%CrI 5.1–67.0%) from female to male per partnership (35), which was similar to the earlier estimates (36).

2.1.1.3 Geographic distribution

Based on various methodologies, the number of new chlamydia cases has been estimated since 1995 at which point it was 89 million cases among adults annually (1, 37). Globally in 2016, there were an estimated 127.2 million (95% UI: 95.1–165.9) new chlamydia cases (37). The majority of new chlamydia infections were estimated in upper-middle-income countries (47%) and lower-middle-income countries (31%), followed by low-income countries (13%) and high-income countries (8%) (37). In the European Union and European Economic Area (EU/EEA), overall chlamydia trends stabilized during 2009-2018, with 146 cases per 100 000 reported in 2018 (31).

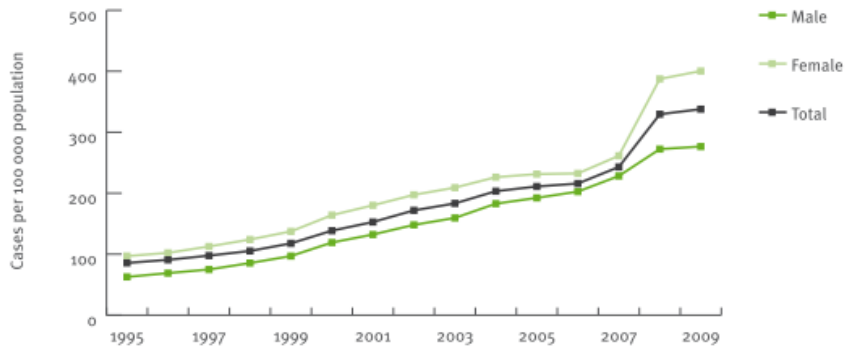
The highest estimated prevalence in the World Health Organization (WHO) regions in 2016 was reported among women in the Region of the Americas (7.0%), followed by the African Region (5.0%) and the Western Pacific Region (4.3%) (***Table 1***). Among men in 2016, the highest chlamydia prevalence was estimated in the African region (4.0%), followed by the Region of the Americas (3.7%) and the Western Pacific Region (3.4%)

Table 1. WHO regional prevalence estimates (with 95% uncertainty intervals) of chlamydia 2012 and 2016 by sex (source: reference (37), reproduced with permission).

WHO Region, by sex	Chlamydia	
	2012	2016
Women		
African Region	3.7 (2.7–5.2)	5.0 (3.8–6.6)
Region of the Americas	7.6 (6.7–8.7)	7.0 (5.8–8.3)
South-East Asia Region	1.8 (1.4–2.2)	1.5 (1.0–2.5)
European Region	2.2 (1.6–2.9)	3.2 (2.5–4.2)
Eastern Mediterranean Region	3.5 (2.4–5.0)	3.8 (2.6–5.4)
Western Pacific Region	6.2 (5.1–7.5)	4.3 (3.0–5.9)
Global total	4.2 (3.7–4.7)	3.8 (3.3–4.5)
Men		
African Region	2.5 (1.7–3.6)	4.0 (2.4–6.1)
Region of the Americas	1.8 (1.3–2.6)	3.7 (2.1–5.5)
South-East Asia Region	1.3 (0.9–1.8)	1.2 (0.6–2.1)
European Region	1.5 (0.9–2.6)	2.2 (1.5–3.0)
Eastern Mediterranean Region	2.7 (1.6–4.3)	3.0 (1.7–4.8)
Western Pacific Region	5.2 (3.4–7.2)	3.4 (2.0–5.3)
Global total	2.7 (2.0–3.6)	2.7 (1.9–3.7)

2.1.1.4 Time trends

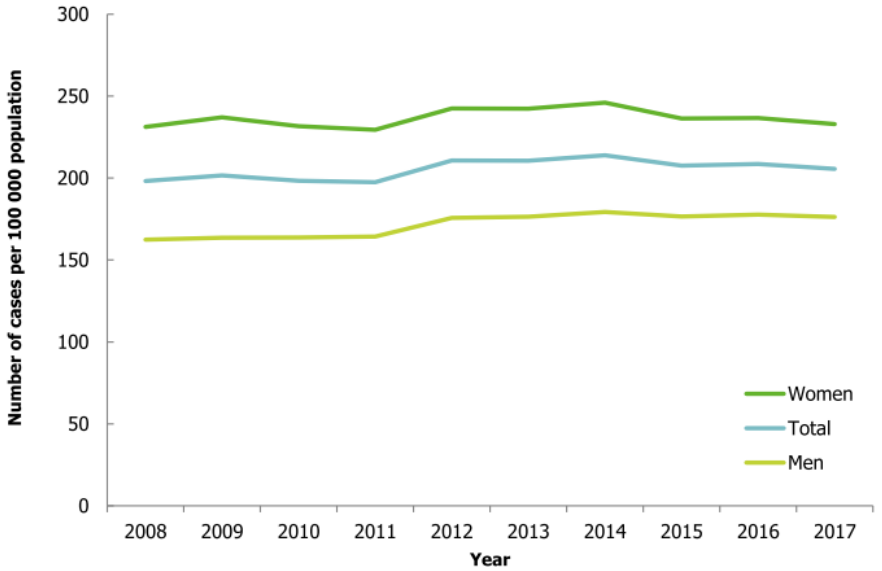
Systematically collected data on chlamydia cases have become available since the late 1980s or the early 1990s, with introduction of surveillance systems elsewhere (38). From late 1980s chlamydia notification rates have decreased in some of the countries (32, 39, 40), while increasing in others (41). The reason behind the decrease was suggested by the change in sexual behaviour as a result of the HIV/AIDS campaigns in place at the time (40). The notification rates increased from 55 cases in 1990 to 139 cases per 100 000 in 2000 in EU/EEA (**Figure *1***) (38). The rates in EU/EEA more than doubled between the years 2000 and 2009, reaching 338 cases per 100 000 population in 2009. However, due to the incompleteness of the reported data and differences in the type of surveillance systems, as well as the differences in the control measures, these rates are based on 8 countries reporting consistently (Denmark, Estonia, Finland, Iceland, Ireland, Latvia, Sweden and the UK) (38). Similar increasing trends during that time were reported from the USA, Canada and Australia (41-43). The reason for the increase in chlamydia rates during that time were possibly the introduction of more sensitive diagnostic methods and possibly increased chlamydia prevalence in the population, as well as an alteration of target groups for testing for high-prevalence chlamydia (44-47).



Note: Based on data from eight countries that have reported consistently.
In 2008, UK introduced a new chlamydia surveillance system that collects data from community-based test settings as well as from STI clinics; prior to 2008, data were based on STI clinic diagnoses only.

Figure 1. Number of notified chlamydia cases per 100 000 population in EU/EEA Member States that have reported consistently (n=8), 1995-2009 (source: reference (38), reproduced with permission).

Furthermore, notification trends in EU/EEA between 2008 and 2017 stayed relatively stable, with 198 cases in 2008 and 206 cases per 100 000 in 2017 (***Figure 2***) (48). Reporting have improved since 2008, thus rates since this year are based on 17 countries reporting consistently. Although, the overall chlamydia trend remained stable in EU/EEA during 2008-2017, rates of chlamydia continued to increase in Denmark, Finland and Norway, as well as in North America (48-50). Nevertheless, notification rates should be interpreted with caution as reporting of the cases could be incomplete, the quality of the laboratory tests may vary over time and screening programmes may cover diverse populations (51).



Source: Country reports from Cyprus, Denmark, Estonia, Finland, Iceland, Ireland, Latvia, Lithuania, Luxembourg, Malta, Norway, Poland, Romania, Slovakia, Slovenia, Sweden and the United Kingdom.

Figure 2. Number of notified chlamydia cases per 100 000 population in EU/EEA Member States that have reported consistently (n=17), 2008-2017 (source: reference (48), reproduced with permission).

In Sweden, chlamydia notification rates started to decrease just the after introduction of the surveillance system in 1988; between 1990 and 1994 from 311 cases to 155 cases per 100 000 (***Figure 3)***. Between 1994 and 1997, a plateau was noted with the lowest recorded notification rates. Between 1998 and 2005, chlamydia rates increased in Sweden by 113%. In 2006, a new genetic variant of *Chlamydia trachomatis* (nvCT, new Swedish variant of *C. trachomatis*) was discovered with a deletion in the cryptic plasmid DNA, which was a target for commonly used diagnostic tests in Sweden at the time (52). Thirteen out of 21 counties (62%) in Sweden used the diagnostic tests that were unable to detect nvCT, which led to missed diagnoses possibly as early as in 2003 (53, 54). The proportion of nvCT among detected cases varied from 10% to 65% between Swedish counties in late 2006 and early 2007 (55). The deficient laboratory tests were replaced with adequate assays able to detect nvCT, which allowed detecting accumulated undiagnosed nvCT cases, leading to the increase in the national notification rates of chlamydia in 2007-2008 (56). In addition, the testing for chlamydia and partner notifications were reinforced as well. As a result, the proportion of nvCT among all chlamydia cases dropping to 5% in all counties towards 2015 (57). Notably, only sporadic or no cases of Swedish nvCT were reported elsewhere in the world (58-63). Following the years after the discovery of nvCT, the chlamydia rates dropped in Sweden between 2009 and 2020 from 406 cases to 321 cases per 100 000 (***Figure 3***). Recently, another new variant of *C.trachomatis* was reported to emerge in Finland in 2019 (64). These incidents urge countries to be vigilant in monitoring chlamydia trends and assessing the quality of the laboratory diagnostic tests.

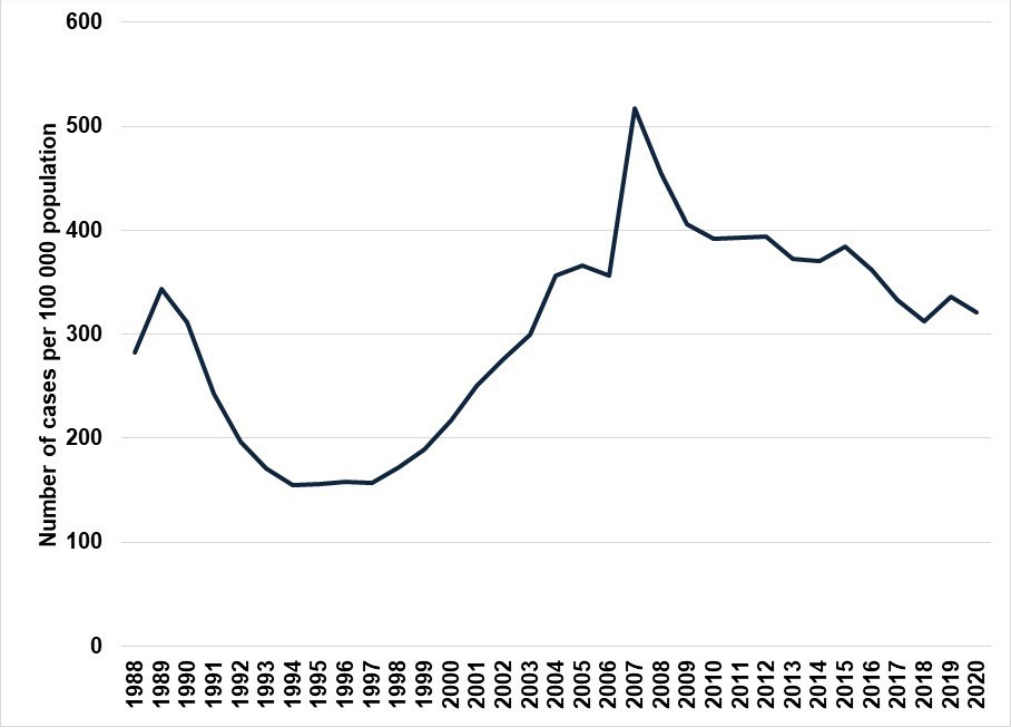


Figure 3. Number of reported chlamydia cases per 100 000 population in Sweden, 1988-2020 (Source: Public Health Agency of Sweden. The data for 2020 is preliminary as per January 14, 2021).

Epidemic phases for sexually transmitted infections were proposed to describe long-term trends of chlamydia (65, 66). As proposed, the first phase starts with the introduction of chlamydia to the susceptible populations, effective transmission through the sexual networks and eventual establishment in the general population. Next, the hyperendemic phase is characterised by rising rates of infection and introduction of the control measures to reduce incidence and prevalence. After control measures to reduce the transmission probability of chlamydia or its duration of infectiousness are in place, chlamydia trends are expected to fall as prevalence declines and as a result, the decline phase begins (66).

2.1.1.5 Demographics

Globally, the estimated number of new cases in 2016 was 34 cases per 1 000 women (95% UI: 25–45) and 33 per 1 000 men (95% UI: 21–48) (37). For instance, in the USA, around 97% and 94% of all chlamydia cases were reported in 2018 in the age group 15-44 years in women and men respectively, with male-to-female ratio of 0.7 (49). Similarly in EU/EEA, the majority of the cases are reported in the age group 15–24 years (62% of cases with known age), followed by the age group 25-34 years (27% of the cases), with male-to-female ratio of 0.8 (31). Women are overrepresented in the number of chlamydia cases most likely due to health-care seeking behaviour, e.g., more frequent contacts with health care services due to routine visits with gynaecologists and family planning counselling. In Sweden, 85% of chlamydia cases are reported in the individuals aged 15-29 year. The highest reporting rates among women are in the age groups 15-19 and 20-24 years (***Figure 4***). Among men, the highest reporting rates are notified in the age group 20-24 and 25-29 years. Overall, 57% of all notified cases were reported among women between 1992 and 2019 in Sweden. The male-to-female ratio of 0.8 has been unchanged since 2001 (32).

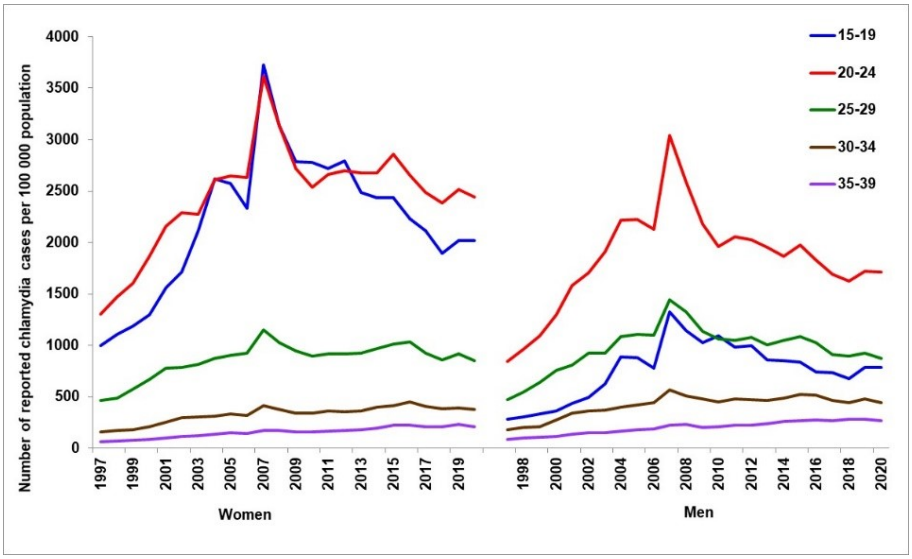


Figure 4. Number of notified chlamydia cases per 100 000 population in Sweden by age groups and sex, 1997-2020 (source: Public Health Agency of Sweden. The data for 2020 is preliminary as per January 14, 2021).

2.1.1.6 Clearance of chlamydia infection

Chlamydia infection can clear spontaneously without treatment as a result of the immune response (67). The duration of untreated chlamydia infection is an important factor to account for when considering measures to control chlamydia in the population. Studies among women reported that asymptomatic chlamydia has cleared naturally in approximately 50% of the women at the 1 year follow-up (68, 69), in 82% of the women the infection cleared after 2 years and 94% of the infection had cleared after 4 years of follow-up (68). It was also reported that women with persisting infection at enrolment had a five times higher reinfection rate at the follow-up visit (OR=5.2, 95%CI: 1.2–33.0); as compared to women with spontaneous resolution at enrolment (the time when patients who had tested positive for chlamydia returned for treatment) (70). The bacterial load was reported to be stable or decreasing during the first month after a positive chlamydia test in the majority (90%) of the patients at STI clinics (71). In the modelling study it was estimated that duration of asymptomatic infection in women was 1.36 years (95%CI: 1.13-1.63 years) (72). In a recent study at STI clinics, it was reported that women infected with chlamydia at a single anatomic site cleared a viable chlamydia infection at a higher rate (follow-up of median 9 days) than women concurrently diagnosed with rectal chlamydia (73). Studies in men reported higher proportion of persistent infections and slower clearance than in women (74, 75). The mean infection duration time in men was estimated to be 2.84 (95%CrI: 0.87-18.79) years compared to 1.35 (95%CrI: 1.13-1.63) years in women (74). In a population of STI clinics, between 7% and 57% of the chlamydia infections cleared between testing and treatment time (median of 10 days), depending on anatomical site sample (urogenital, anorectal, pharyngeal) and subgroups of population (heterosexual vs MSM) (76). In the same study authors reported that lower bacterial load at the initial testing was associated with clearance in all types of samples (76).

2.1.1.7 Risk factors for infection

A lot of research has been done on factors associated with chlamydia acquisition over the past 30 years. Studies that covered different populations, such as health care clinic patients, the general population, and users of a website for ordering home-based chlamydia sampling kits, pregnant women and military recruits were hence subject to different types of biases. Well-established independent factors associated with chlamydia are: younger age at the first intercourse, age under 25 years, multiple sexual partners within during the previous year and during lifetime as well as recent change of partner, inconsistent condom use with casual or new partners and the occurrence of previous STIs (77-84). Some studies also identified relationship between socio-economic status and chlamydia (85), however results are inconclusive and it was suggested that the neighbourhood poverty level should be used instead of socio-economic status as potentially better measure (86, 87). Alcohol and drug use as markers of risk-taking behaviour were also reported to be associated with chlamydia (83, 85, 88-90). Countries, where it is possible to study ethnicity or migration status, also found an increased risk to contract chlamydia (87, 91-94). In return, correct and consistent condom use is reported to be associated

with reduced a risk for chlamydia (95). Several studies also investigated association between use of oral contraceptive pills among women and risk for chlamydia, but provided inconclusive evidence so far (96). Recent studies also looked at the emerged potential contributors of increased risk for chlamydia, such as digital dating apps, however no association was reported (97).

2.1.1.8 Risk factors for repeated infection

Repeated chlamydial infections are also common and are results of a failure of the antibiotic treatment or from reinfection due to unprotected sexual contact with either an untreated existing sexual partner or a new infected sexual partner (98-101). The aim of actively promoted testing (screening) is to detect and treat infections, reduce further transmission and prevent the sequalae of untreated chlamydia infections (102). The rate of re-infection is found to be 29.9 episodes/100 person-years in UK (101), 23.6 episodes/100 person-years in Denver, USA (100), and 34 episodes/100 person-years in Indianapolis, USA (98). The rate of reinfection is known to be health care facility-, age- and sex- dependant with highest rate among those under 20 years of age and among females (100). The risk of reinfection with chlamydia decreases with increasing age (e.g., infections is twice as high among those females < 16 years compared to with women aged 20-21 years) (103).

In a recent review the median proportion of females re-infected with chlamydia was 13.9% (range: 0%–32%) and among males the median reinfection rate was 11.3% (range: 9.8%–18.3%) (102, 104). Among females, younger age is reported consistently statistically associated with a higher risk of reinfection (102, 103), some studies report a relationship between race and ethnicity as well (102). In some studies in females, it is demonstrated that a co-infection with gonorrhoea increased the reinfection with chlamydia (102). Among males, a history of STIs was consistently predictive of reinfection with chlamydia, as well as younger age and non-white race (104). High-risk sexual behaviour (incl. not using condoms), change in partners and higher number of sexual partners consistently are associated with increased risk of reinfection with chlamydia among males and females (102, 104), which also could be linked to “core transmitters” (105). Core groups are defined as a smaller proportion of the individuals who acquire STIs frequently and transmit them, and thus sustaining the transmission of STIs (105, 106). Therefore, it is important that the identification and awareness of high-risk individuals is continuously updated. In Sweden, at the time of the beginning of this thesis project, no data was available on re-infection. As a result, a prospective cohort study was initiated in 2007 in an STI drop-in clinic in Stockholm (107), which Studies I and II are based on.

2.1.2 Prevalence

It is possible to identify risk groups for chlamydia via monitoring the positivity rate defined as the proportion of positive for chlamydia among individuals tested for chlamydia. This measure is used often as a substitute for prevalence (proportion of positive for chlamydia in the population in a given time), since prevalence estimates might not be available due to cost demanding studies. Positivity rates are regularly evaluated in countries with screening programmes (108-111). For instance, in the UK`s screening programme, the positivity rate is found to be at its peak at the age of 18 for women with gradual decline, and peak at 19 years of age for men, remaining stable up to 24 years of age, and it was found to mirror well the reported prevalence (111). Similarly, the positivity rate was reported to approximate well the prevalence in other countries (112, 113). However, the increasing proportion of repeated testing and repeated chlamydia infections can affect the positivity rate, and as a result overestimate the actual prevalence (114).

In Sweden, we lack population-based chlamydia prevalence estimates. As a possible approximation for the prevalence, we use positivity rate among the tested general population. Overall, the positivity rate among men is higher than in women through all years even though many fewer men are tested for chlamydia: only 30% of all tested individuals are men (***Figure 5***). Positivity rate was at its highest in 2007: 8.9% in women and 14.2% in men, which is related to the catch-up of nvCT discovered in Sweden in 2006. Since 2010, the positivity rate is declining continuously in both sexes, while the number of persons tested is increasing (***Figure 5***).

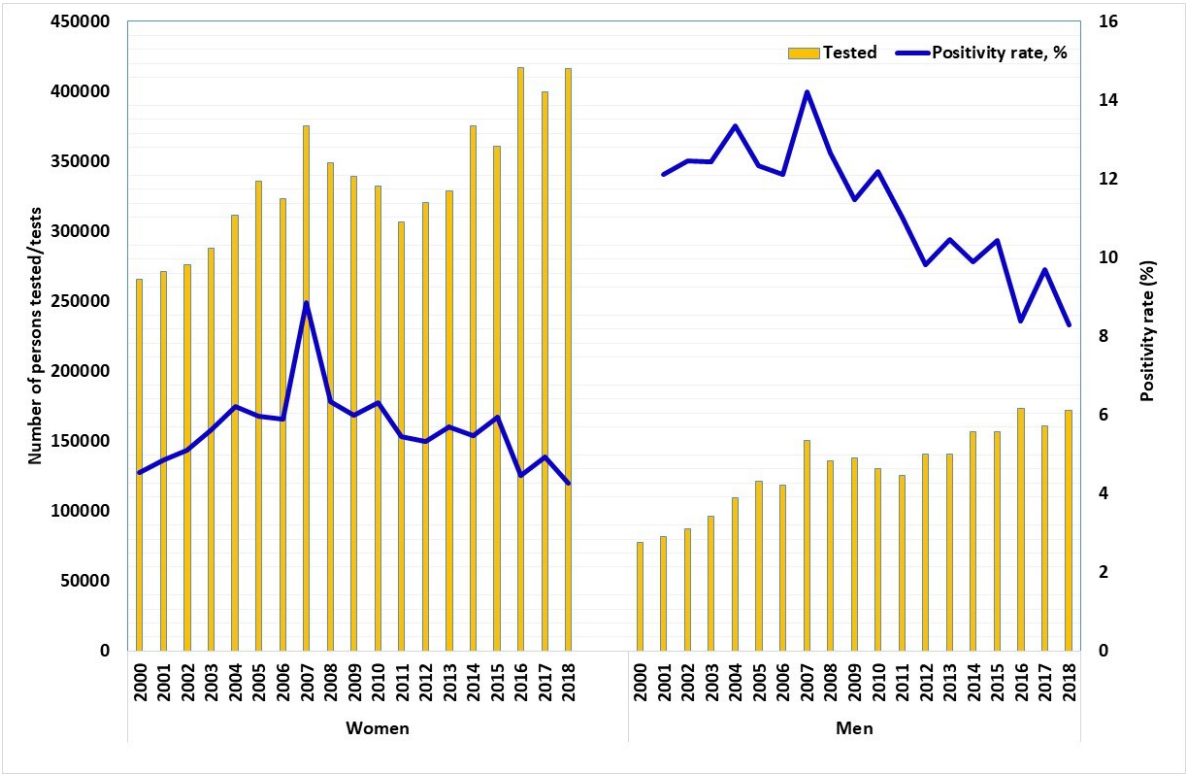


Figure 5. Number of persons tested/tests for chlamydia and positivity rate (%) by sex in Sweden, 2000-2018 (source: Public Health Agency of Sweden).

Another approach to monitor the effect of control measures is to estimate/measure the actual prevalence of chlamydia in the general population. Several European countries were able to measure population-based prevalence in the most affected population of 15-29 years of age (79, 84, 115, 116). Recent global prevalence estimates (2016) reported a chlamydia prevalence of 3.8% (95% uncertainty interval, UI: 3.3–4.5) in women and 2.7% (95% UI: 1.9–3.7) in men (37), which was also similar to another recent meta-analysis study (117). Both studies found higher chlamydia prevalence in the region of the Americas, the African region, followed by the Western Pacific region and the Eastern Mediterranean region (37, 117). In the countries of the European Union alone, the chlamydia prevalence (pooled average) in individuals under 26 year old was estimated as follows: 3.5% (95% CI 1.9 - 5.2%) among men and 3.6% (95% CI 2.4 - 4.8%) among women, with a limitation of participation bias due to low response rate in included studies (118).

Efforts to measure the prevalence in biological samples were done over the years in selected populations in Sweden as well, such as among visitors of STI clinics and individuals ordering chlamydia tests online (78, 119, 120); hence, those studies are prone to selection and participation bias (118). Other possible sources of the information on chlamydia prevalence could be used from the national behavioural surveys, which collect data on sexual risk behaviour and self-reported chlamydia prevalence (121-123). For instance, in the latest knowledge, attitude and behaviour survey UngKAB-2015, self-reported lifetime chlamydia infection among 20-24 year old women was 14.7% (95%CI: 12.7 – 16.8) and among men 6.8% (95%CI: 5.0 – 8.6); among 25-29 year old women was 21.1% (95%CI: 18.6 – 23.5) and among men 11.0% (95%CI: 8.8 – 13.2) (122). However, several methodological limitations were reported in such surveys (124, 125). Besides, self-reported chlamydia could be biased also due to the recall bias. Therefore, we initiated a study to estimate the population-based chlamydia prevalence (Study IV).

2.1.3 Sequelae

In women, untreated chlamydia infections can ascend the upper genital tract and affect the reproductive tract causing sequelae, such as pelvic inflammatory disease (PID), ectopic pregnancy (EP) and tubal factor infertility (TFI) (72, 126-128). From the summary of the studies performed in the 1990-s, it was estimated that 30% of acute PID can be linked to an chlamydia infection, but other possible pathogens (*Neisseria gonorrhoeae, Mycoplasma genitalium*) have a potential to cause PID as well (129). In the POPI (prevention of pelvic infection) trial in England it was found that 1.6% of women positive for chlamydia at the baseline of the intervention arm (screened and treated) developed PID, as compared to 9.5% positive for chlamydia in controls at baseline (deferred screening) during the 12 months follow-up, resulting in RR=0.17 (95%CI: 0.03–1.01) (130). A more recent study in the United Kingdom estimated that 20% (95%CrI: 6%–38%) of PID, 5% (95% CrI: 1%–12%) of EP and 29% (95%CrI: 9%–56%) of TFI are attributed to a *Chlamydia trachomatis* infection in women aged 16–44 years (131). Record linkage studies in Australia reported that during a follow-up

after approximately 10 years, women with chlamydia infection had a RR=1.8 (95%CI: 1.6–1.9) of PID and a RR=1.4 (95%CI: 1.2–1.7) of EP compared to women who tested negative for chlamydia (127, 132). Recent estimates suggest that approximately 15% (95%CrI: 5%–25%) of an untreated chlamydia infection progresses to a clinical PID (72), while earlier estimates showed that up to 40% of untreated chlamydial or gonococcal infection in women can develop into PID (133). On the other hand, a study based on the data from the Netherlands estimated that 0.43% progress to a clinical PID, 0.07% cause ectopic pregnancy, and 0.02% causes tubal factor infertility in women with a current chlamydia infection (134). Thus, authors concluded that earlier assumptions regarding sequelae were overestimated, thus, cost-effectiveness of the screening programmes could be considered not favourable (134).

A recent review also reported a significant relationship between chlamydia and EP (OR=3.03; 95%CI: 2.37–3.89) (126). Another review also found a relationship between chlamydia infection and pregnancy harms, such as increased risk for spontaneous abortion (RR=1.5; 95%CI: 1.2–1.9) and stillbirth (RR=1.3; 95%CI: 1.1–1.5), and this consequence was more pronounced in low- and middle-income countries in comparison with high-income countries (135). However, since these estimates are produced based on studies that used various kinds of methodologies (randomized trials, observational studies) and populations, there are challenges in ascertaining the sequelae since the timing of the PID and other long-term sequelae are still debatable and the imprecision of the estimates and biases should therefore be considered (2, 136).

Studies also reported that an increasing number of repeated chlamydia infections are associated with increased odds for PID (137, 138). For instance, two chlamydia infections had OR=4.0 (95%CI: 1.6–9.9), while three or more chlamydia infections had OR=6.4 (95%CI: 2.2–18.4) for PID (137).

Although much attention had been paid to the sequelae in women, there are also suggested sequelae in men due to chlamydia infection, such as chronic prostatitis/chronic pelvic pain syndrome and epididymitis, which can affect spermatogenesis and fertility (139-141). Nevertheless, the role of chlamydia in male infertility is still debatable (142).

3 CHLAMYDIA CONTROL AND PREVENTION IN SWEDEN

3.1.1 Surveillance system

Chlamydia infection is a mandatory notifiable disease in Sweden since 1988 according to the Communicable Disease Act (143). However, testing for chlamydia has been available since the early 1980s, with reporting from the laboratories starting already in 1982. Currently, a commonly agreed case definition within the EU is used for reporting chlamydia to the international and national level (144). The chlamydia surveillance system is case-based, passive and universal. The electronic surveillance system SmiNet-2 is operating since 2004, with all laboratories and health-care clinics having access to report cases (145). Each laboratory report verified chlamydia case from any anatomical site to the system electronically, as well as do clinicians (***Figure 6***). All residents in Sweden have an unique personal identification number (PIN), which is used for the reporting to SmiNet-2 (146). However, notifications of STIs and HIV do not contain full identity; instead, a shortened identification number (Swedish, *rikskod*) is used for reporting. This number is not unique any longer, which prevents the automatic linkage of the laboratory notification with the clinical notification. Therefore, only the clinical chlamydia notifications are used for the official statistics. The system is accessible to County Medical Offices (their county-specific data only) and the Public Health Agency of Sweden, which has access to all data on a national level. The system was evaluated for the period 1997-2008, and was found to provide accurate and relevant data for action (147).

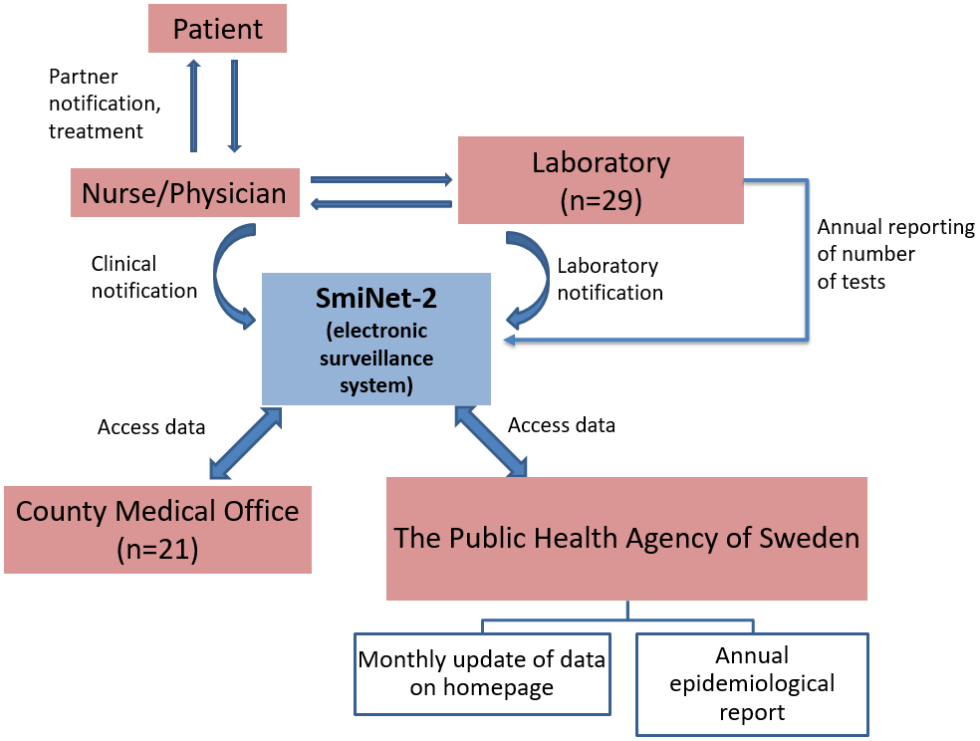


Figure 6. Schematic presentation of electronic surveillance system in Sweden.

3.1.2 Opportunistic testing

As part of the secondary prevention (**Figure** *7*), testing for chlamydia (as for gonorrhoea, syphilis and HIV) in Sweden is available free of charge for everyone who wishes at all health-care facilities and it is covered by the County Council budget. Current (2015) national guidelines recommend chlamydia testing for anyone who had new or unprotected sexual contacts during the past year (148). Individuals should be offered testing for chlamydia in any health care facility as part of the opportunistic testing (opportunistic screening) strategy in Sweden. Asymptomatic persons could test themselves as early as 7–10 days after an unprotected sexual contact. However, regional guidelines for chlamydia testing can be more explicit; for instance, in the Skåne Region, individuals, who previously had a chlamydia infection and/or gonorrhoea, are considered at higher risk, and are recommended to be offered testing for chlamydia (149). Another example is the Västerbotten Region, where re-testing for chlamydia 2 months after the initial chlamydia infection is explicitly recommended (150). Specialized clinics are performing most of the chlamydia testing: STI clinics and youth health clinics (65% of all notified cases in 2019), gynaecology, maternal care and family planning clinics (12% of the cases) (151). General practices could be a valuable asset for the STI testing, although they reported only 13% of the total cases in 2019. Efforts were made to increase engagement of general practices in chlamydia testing in Sweden (152). During 1992-2018, almost 12 million tests were performed in Sweden (32). Of those tests, 77% were performed in women during the period of 1992-2004 as in contrast to 68% during the period of 2009-2018 (153). On average, 66% of all tests were reported in the age group 15-29 years (data available for 2009-2018) (153).

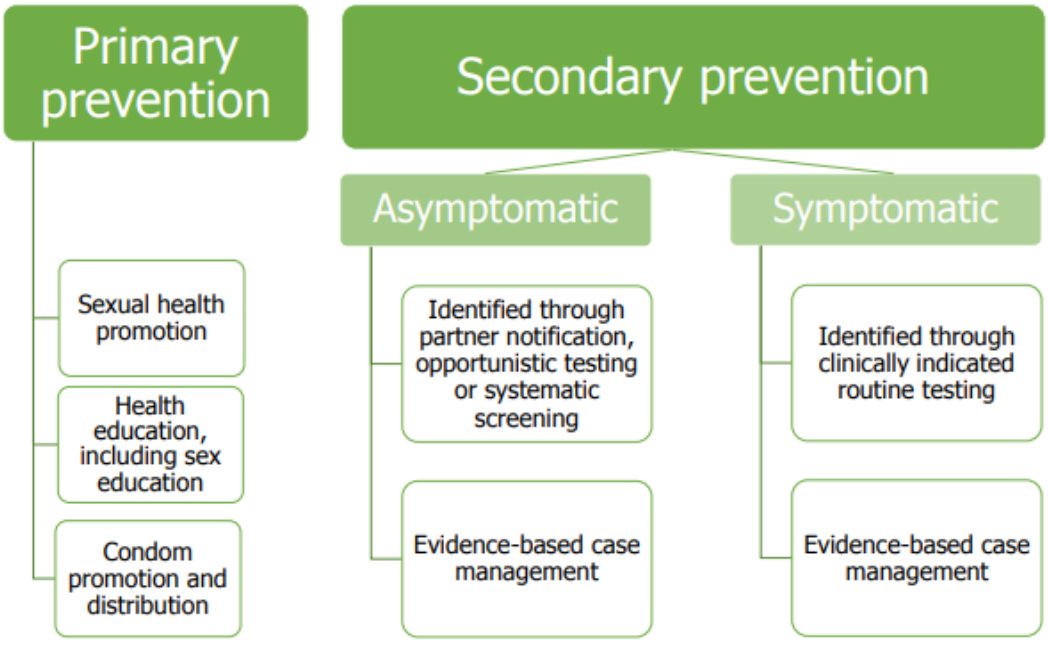


Figure 7. Interventions for chlamydia control (image source from reference (154), reproduced with permission).

In the beginning of 2000s, online services started to develop in Sweden (contracted by the County Councils) offering self-samples for chlamydia at individual´s homes (155). The operation of such testing is feasible due to a unique PIN. The sampling kit is sent home for self-sampling, and is then returned to the laboratory for the testing. The results of the tests are obtained online by using the PIN. In case of a positive result, patient are referred for clinical management and partner notification. Simultaneously, positive findings are reported electronically to the surveillance system SmiNet-2. Currently, all counties (n=21) in Sweden offer such online services through various providers as for example the 1177 Vårdguiden (national health guidance service). The data on number of tests ordered exclusively online are not routinely collected at the national level. Nevertheless, a recent study evaluated online-based testing, performed in 14 counties, found that the proportion of such tests increased from 11% in 2013 to 18% in 2017 (156). This study also found that internet-based testing had a more substantial increase among women (by 115%) than among men (by 71%) during the study period of 2013–2017.

Opportunistic testing as part of the control strategy (**Figure 7**) was never formally evaluated in Sweden. Several studies reported limited evidence of effectiveness and cost-effectiveness of opportunistic screening on averted sequelae and chlamydia prevalence (157-159). A systematic screening programme was piloted for 3 years in the Netherlands, and was discontinued due to lack of effectiveness (160). Currently, only England (UK) has a systematic screening programme in place (161).

3.1.3 Laboratory diagnostic methods

Laboratory methods for the identification of *Chlamydia trachomatis* have developed over the years and comprise both direct and indirect methods. The direct methods include cell culture, which was considered to be a golden standard (162). However, the culture method is dependent on the correct specimen collection, storage and transportation. The sensitivity of the culture method is less than 75%, however, the specificity is high (up to 100%) (163). Other various non-amplification methods, which were based on antigen detection and nucleic acid hybridization methods are no longer considered up to date. These methods had a sensitivity between 50%–75% (164), and no longer considered recommended methods for chlamydia diagnostics. Nucleic acid amplification tests (NAATs) came to use in Sweden in the late 1990s, and are based on the amplification of nucleic acid sequences of the pathogen. Due to the high sensitivity, specificity, and considerable speed of diagnosis, NAATs became a method of choice and is currently considered a golden standard method (165). Various commercial NAATs are available in the market, and their performance is depending on the sample type used for diagnostic. In women, sensitivity of NAATs was estimated above 93%-95% for first-void urine specimens, above 97% for clinician– and self-collected vaginal swabs, above 91%-93% for endocervical swabs (166, 167). The specificity was estimated above 99% for all female urogenital specimen types (166, 167). In men, sensitivity for first-void urine specimens was estimated 99%-100%, while specificity above 98% (166, 167). In women, due to the relatively

lower sensitivity for urine specimens, vaginal swabs are the preferred specimen, which have similar sensitivity and specificity to cervical swabs (165, 168). For pharyngeal, rectal and conjunctival specimens, NAATs can be used, but their lower sensitivity and specificity should be considered for these type of specimens (165). Recently two NAATs were approved by the Food and Drug administration Agency in the USA for diagnostic testing of extragenital specimens (169).

Indirect diagnostic methods, such as serological testing, is not used for the diagnosis of chlamydia as the method cannot discriminate between current and past infection. However, serology continue to be used in epidemiological research to study chlamydia prevalence and possible correlation with infertility in women (170-173).

In addition to the laboratory-based tests, rapid tests at the point of care (POC tests) become more attractive for the users as results are available in a short time (approx. 30 min) (163). The advantage of these tests is also, that patients can receive counselling and treatment without any delay (163). However, in a recent systematic review, the authors reported the sensitivity of POC tests to be 48% and specificity 98%, which hinders to recommend these assessed tests for the triage of urogenital chlamydia (163).

3.1.4 Treatment

In Sweden, uncomplicated chlamydia can be treated at health care facility where it was diagnosed, while complicated chlamydia cases are referred to specialized STI clinics. Treatment guidelines in Sweden (148) are in line with European guidelines (165), with recommended first-line treatment is Doxycycline for urogenital uncomplicated chlamydia. Alternatively, treatment with Azithromycin could be prescribed if the first-line treatment is not possible, and the diagnosis with *Mycoplasma genitalium* is ruled out (148). This is due to the resistance development by *M.genitalium* against Azithromycin (174). True antimicrobial resistance of *C.trachomatis* is rare; however, treatment failures have been reported, most likely due to poor compliance, tolerance to treatment, or reinfection (175, 176). Test of cure is not routinely recommended for uncomplicated chlamydia cases, but could be done if there is a suspicion of non-compliance to the treatment or the first-line treatment is not used. Expedited partner therapy (patient delivered partner therapy) is not a praxis in Sweden, since treatment has to be pathogen-based and requires laboratory testing before treatment initiation (148). In some countries, expedited partner therapy is permitted and could be beneficial to reach partners, and reduce the transmission of chlamydia (33, 165).

3.1.5 Partner notification

Partner notification (PN, partner referral, contact tracing) is a process of identifying contacts of index chlamydia cases and is part of a control programme in Sweden, legally

regulated by the Communicable Diseases Act (143). Partner notification has important effects both on an individual level (by preventing re-infection of index case) and at the population level (by reducing the transmission of the chlamydia infection) (177). Several modelling studies suggest that PN (together with screening) strategies are effective tools in averting chlamydia cases and reducing chlamydia prevalence and could be cost-effective with some scenarios (178, 179).

International guidelines recommend either provider referral or patient referral to reach contacts 2 to 6 months back in time (33, 180). In Sweden, the Communicable Diseases Act indicates that contact tracing (Swedish, *smittspårning*) has to be performed by specially trained staff, for instance, social workers and midwifes (143). The same law defines the obligation of the index case and his or her contacts to participate in the partner notification process. The evidence suggests (181) that partner notification should be carried out to up to 12 months back in time, which is also recommended for the health care providers (182). Local county-specific guidelines recommend partner notification 6 to up to 12 months back in time. In Sweden, several counties have a centralized contact tracing service, where index chlamydia cases can be referred for partner notification (183). This approach showed better results in reaching partners as compared to the single individual health care providers (not specialized in partner notification) (181, 184). Quality indicators were suggested on the partner notification process (e.g. how soon the partner notification started and finished, how far back in time it was performed etc.), and are important to follow up on by health care clinics and County Medical offices (182).

3.1.6 Primary prevention

Controls on chlamydia infection through the primary prevention (***Figure 7***) activities include sexuality education in the school curriculum, which is compulsory in Sweden since 1955 (185). Sexual health promotion is a key component, where several actors, in addition to the schools, are involved. One of the non-governmental organizations that has been particularly involved is the Swedish Association for Sexuality Education (Riksförbundet för Sexuell Upplysning - RFSU) (186), who started prevention work as early as 1933. The network of youth health clinics across the country is in addition to providing medical care, also offering counselling on safe sex, mental health, and prevention of alcohol and drug use for individuals aged 13-25 years. They are also possible to interact with through their websites (187) and through social media. Youth health clinics were introduced in the 1970s and have grown in number, reaching 250 facilities in 2018 (188). All these actors are important collaborators to fulfil objectives of the National Action plan for chlamydia prevention launched in 2009 (189). The main themes to pursue by the plan were to increase condom use with casual partners, the understanding of consequence of unsafe sex and to increase the number of persons tested for chlamydia. Additionally, through the years, information campaigns promoting condom use and testing were run by governmental and non-governmental organizations at the national and regional level. One such initiative has

been in place since the early 2000s, called Chlamydia Monday, promoting testing for chlamydia. The campaign was shown to be a cost-effective initiative (2007) (190).

As part of the primary prevention, vaccines could be an effective tool in controlling STIs. Because of the highly effective vaccines for human papillomavirus infection, a remarkable decrease in HPV infections (191) has been seen, with the potential to eliminate cervical cancer (192). Modelling studies have suggested that a potentially effective chlamydia vaccine could control and even eliminate chlamydia infections (193-195). Intensive research is currently going on, with the hope of finding a chlamydia vaccine in the near future (196).

4 RESEARCH AIMS

The aim of this thesis work is to gain enhanced understanding on chlamydia epidemiology in Sweden.

The specific aims of the studies are as follows:

Study I: To investigate how sexual behaviour, testing behaviour and demographic factors are associated with chlamydia infection diagnosis.

Study II: To identify unobserved groups (latent classes) based on sexual behaviour and substance use patterns; and study how latent class membership is associated with repeated chlamydia testing and repeated chlamydia infection.

Study III: To examine and compare chlamydia infection notification trends before and after emergence of the new variant of *Chlamydia trachomatis* (nvCT) in Sweden. Additionally, to study the association between testing for chlamydia and chlamydia notification rates prior and after emergence of the nvCT.

Study IV: To estimate population-based *Chlamydia trachomatis* prevalence in Sweden.

5 MATERIALS AND METHODS

5.1 OVERVIEW

The overview of the aims and study designs are presented in ***Table 2***.

Table 2. Overview of study designs included in the thesis.

	Study I	**Study II**	**Study III**	**Study IV**
Study outcome	Association of demographics, sexual and testing behaviour with chlamydia diagnosis.	Latent classes based on sexual behaviour and substance use patterns. Association of latent classes with repeated testing and chlamydia infection.	Trend and cyclic component before and after emergence of the nvCT in Sweden. Association between testing and chlamydia rates before and after emergence of the nvCT.	Chlamydia prevalence and incidence rates
Study design	Cohort (cross-sectional)		Population-based longitudinal	Population-based mathematical modelling
Study population	Men and women aged 20–40 years attending an urban STI-clinic		Total Swedish population	Men and women aged 15-29 years living in Sweden
Statistical and mathematical analysis	Log- binomial and Poisson regression	Latent class analysis, ordinal and multinomial logistic regression	Time series analysis with negative binomial regression	Compartmental deterministic mathematical model

5.2 DATA SOURCES

5.2.1 Cohort study (Study I and II)

A prospective cohort of visitors to a drop-in STI clinic in Stockholm was recruited between December 2007 and June 2008 (107). The clinic served the population aged 20 years and older. Annually approximately 15 000 visits were made, of those 60% by men (107). Inclusion criteria in the study were as follows: 20–40 years of age, accepting to answer a paper questionnaire before testing for chlamydia, and accepting to link with the subsequent *Chlamydia trachomatis* laboratory testing result. All visitors presenting for chlamydia testing, irrespective of symptom presence, were consecutively invited to take part in the study.

5.2.1.1 Exposures

The questionnaire consisted of several parts, including the following themes: 1) demographic background (gender, age); 2) testing for and having had STIs; 3) sexual experiences/behaviour (e.g. number of sexual partners during the past 12 months, current steady relationship, condom use with new or casual partners); 4) substance use (alcohol and drug use during the past 6 months); and 5) gender-specific exposures: for men – getting a woman unintentionally pregnant, and for women – having used emergency contraceptive pills (107). Information on presenting symptoms was extracted from a case report form by health-care staff at the clinic. All abovementioned variables were considered as exposures in Study I. For Study II, fewer number (n=9) of variables were included into latent class membership analysis, such as reason for the testing, current steady relationship and concurrent sexual partnerships, number of sexual partners during the previous 12 months, type of last sexual partner, condom use and responsibility with new/casual partners, alcohol and drug use during the previous 6 months, and self-assessed alcohol impact.

5.2.1.2 Outcomes

The outcome of Study I was *Chlamydia trachomatis* diagnosis (positive or negative) verified by DNA amplification assay (ProbeTec™ by Becton-Dickinson, USA) from the samples provided by study participants; from women, self-collected vaginal swab put into first void urine, and from men, urine samples. All chlamydia-positive individuals received treatment free of charge according to the established treatment protocol. Chlamydia patients also underwent a mandatory partner notification process.

We had two outcomes in Study – II: repeated testing and being infected repeatedly with chlamydia. For each outcome, we looked at short-term and long-term measures. For the short-term testing outcome we used repeated testing for chlamydia during the past 12 months (no/yes) and for the long-term testing we used repeated lifetime testing for chlamydia (no, 1-3 times, 4 or more times). Accordingly, for chlamydia infection short-term outcome we looked at current chlamydia test result (negative/positive) at the time of the study and for long-term

outcome: lifetime chlamydia infection (never, once, twice or more). No and never were considered as reference levels in all outcome analyses.

5.2.2 Infectious diseases register SmiNet-2 (Study III and IV)

5.2.2.1 Data on incident Chlamydia trachomatis cases

We retrieved cases of *Chlamydia trachomatis* infection from the national population-based register on mandatory reportable infectious diseases (SmiNet-2) at the Public Health Agency of Sweden (PHAS) (145). All laboratory confirmed cases of chlamydia from any site of the body (urogenital, pharyngeal and anal samples) are mandatory notifiable in Sweden (143). Ninety-five to ninety-seven percent of reported chlamydia cases were acquired via sexual contact, with most of the remaining cases having no information on the route of transmission, and very few cases acquired via vertical transmission (0.05–0.1%).

For Study III, we aggregated chlamydia cases by 4-week months (here called a "month"), resulting in 13 months of equal length per year (also called the Equal Month calendar), except for extra days in week 53 in some years, which were added to the last week of month-13. From the information available on the cases, we were able to aggregate cases by county group, based on their ability to detect nvCT in 2006 (52, 197): able-to-detect (n=8) and unable-to-detect (n=13). For the yearly analysis, we aggregated chlamydia cases by year to relate to the annual data to number of persons tested. We used mid-year population counts that were obtained from Statistics Sweden by year and county (198). We carried out analyses for two periods: 1992–2004 (before nvCT) and 2009–2018 (after nvCT). We excluded data for the period 2005–2008: 2005–2006 due to potentially missed cases of nvCT; 2007–2008 data were excluded due to a spurious incidence peak and subsequent decrease caused by catch-up of nvCT.

For Study IV, we retrieved reported chlamydia cases and number of cases by *reason for testing* (partner notification, screening or symptoms), and *type of infection* (symptomatic or asymptomatic) from the national register of mandatory notifiable diseases SmiNet-2 (147) at PHAS. We aggregated data by age group (15–19, 20–24, 25–29 years of age), sex and year (2009, 2012, 2015 and 2018). For symptomatic cases, we extracted data on the *number of days* from the onset of the disease to verified diagnosis of chlamydia at the reporting health-care facility. Reported chlamydia cases with missing information on symptomatic or asymptomatic infection were assigned proportionally between both categories assuming that their distribution was the same as the cases with observed information. The same procedure was done for *reason for the testing*, where PN is one of the reasons for testing.

5.2.2.2 Data on testing for Chlamydia trachomatis

For Study III, we used population-based reported annual number of persons tested for chlamydia collected at the PHAS. The number of persons tested are reported from the microbiological laboratories in charge of chlamydia testing in each county, which serve all health care facilities (private and public). For the period 2009–2018, on average 23.4% of counties reported the number of tests performed instead, which could include multiple tests done on the same person at the same testing event (data not available for the first period). As the reported testing data was in aggregated format, it was not possible to eliminate potential re-counts, and we chose to interpret and refer to both types of data as number of persons tested in our analyses. We defined the proportion of persons tested as the number of persons tested (including tests performed) per population aged 15–64 years in each county, being the age interval where most tests are performed. Proportions of tests per population were aggregated nationally and by county group. We used mid-year population counts of ages 15–64 years from Statistics Sweden by year and county (198).

For Study IV, we retrieved number of annual persons tested aggregated by age groups 15–19, 20–24, 25–29 years of age and by sex, collected at PHAS. Data was retrieved for the following years: 2009, 2012, 2015 and 2018.

5.2.3 Other data sources (mathematical modelling, Study IV)

From a cohort study among visitors to a STI clinic, we used data on the *reason for testing* on chlamydia negative persons (120). Thus, we calculated the PN rate for uninfected individuals by sex and for two age groups (20–24 and 25–29) in 2009. We assumed the same PN rate for uninfected as a known quantity for 2012, 2015 and 2018, and for 15–19 years old.

The size of the sexually active population by sex and age group was obtained from the 2015 population-based knowledge, attitude, behaviour survey UngKAB2015 among individuals 16-29 years old (122), using the question on whether the participants ever had sex. We applied the same estimate to all years in our analysis.

Parameters on chlamydia natural history and sensitivity/specificity of the laboratory test were obtained from published literature, as described in Table 2 in the Paper IV. All rates are in units per year.

The population size by age groups and sex was obtained from Statistics Sweden (198).

5.3 STATISTICAL METHODS (STUDIES I-III)

5.3.1 Log-binomial regression and Poisson regression (Study I)

For the dichotomous outcome data logistic regression models are often applied, with estimation of odds ratios (ORs). However, for the frequent outcomes, which are common in cross-sectional studies, the OR can strongly overestimate the relationship. Therefore, for the cohort data other alternative regression models could be applied to estimate risk ratios (RRs), such as log-binomial regression and Poisson regression (199). Both models are generalized linear models and use a log link function to relate predictor variables with the outcome (200). Log-binomial model assumes that dependent variable has Binomial distribution. The maximum likelihood approach is used to obtain estimates and they are reported to have small variances, and are unbiased and efficient (201). However, log-binomial regression models experience a high number of failures, such as non-convergence (202). In Study I, we were able to estimate crude RR associated with chlamydia diagnosis using log-binomial regression. For the multivariable analysis, a log-binomial model with the outcome chlamydia diagnosis had convergence problems, so instead we fitted a multivariable Poisson regression model with robust standard errors estimated through a sandwich estimator (203). The Poisson regression is typically used when the outcome is count data of diagnoses (events) occurring over time, but can be applied to the dichotomous counts (disease/ no disease) as well (204). The Poisson regression model assumes Poisson distribution where the mean is equal to the variance, where the variance increases when the mean increases. Because counts/events are positive numbers, the distribution is highly positively skewed and therefore overdispersion is present (i.e., the variance is greater than the mean) (205).

Due to missing values in our dataset, we created 100 imputed datasets for model building, where plausible values for missing responses were imputed. The implementation of the imputation algorithm for our dataset is described elsewhere (206).

5.3.2 Latent class analysis (Study II)

Latent class analysis (LCA) is a statistical method belonging to finite mixture models. LCA aims to find underlying hidden (latent) groups in the observed population through the set of observed variables (manifest variables). The method is based on estimation of conditional response probabilities and the latent classes (LCs) prevalence using maximum likelihood criterion based on a set of chosen categorical variables (207). Identified patterns amongst a set of given variables produce LCs, where each respondent is assigned to the LC with the highest estimated probability. These LCs are mutually exclusive. The main assumption in the LCA is that observed variables are not highly correlated within the identified LC, known as local independence (208, 209). This analysis approach allows to capture how multiple variables co-occur and interact with each other (a model-based clustering method), as opposite to the conventional regression analysis (variable-oriented analysis), where the association between

independent variable and outcome variable is examined while holding other variables constant. We fitted models with varying numbers (2 to 5) of LCs based on the observed 9 manifest variables (**Figure *8A***). The analysis was stratified by sex. We labelled latent classes based on the item-response probabilities of the respondents.

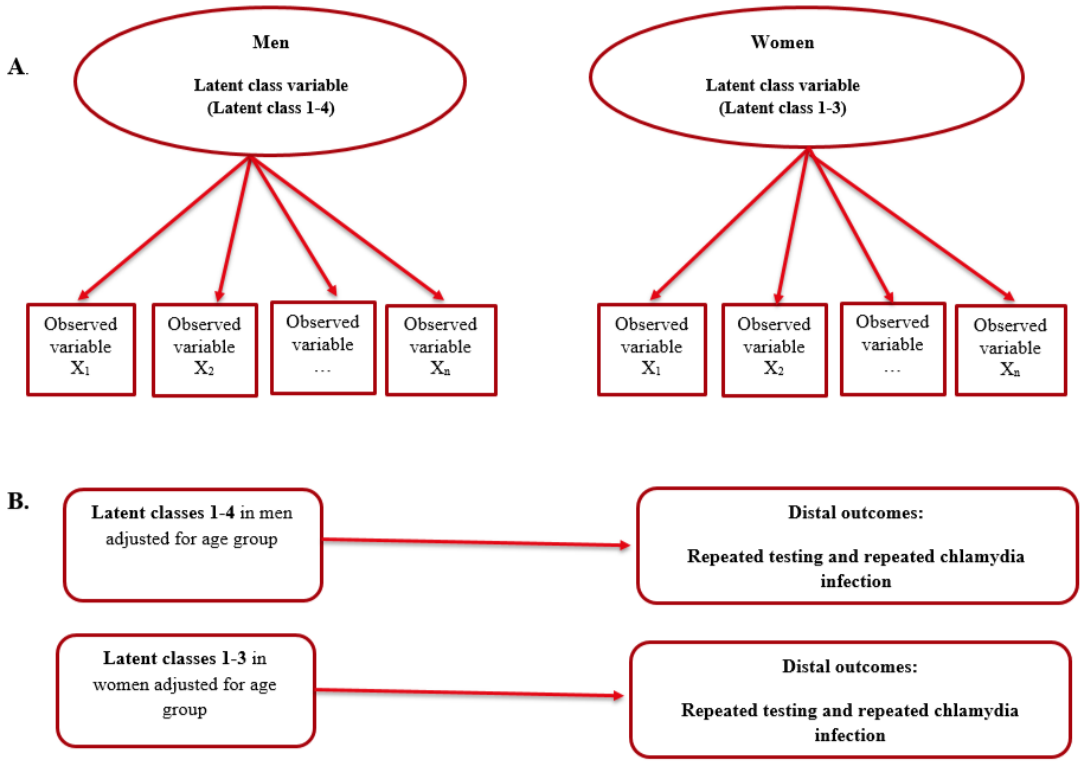


Figure 8. Schematic depiction of analysis steps in latent class analysis study.

5.3.3 Ordinal logistic regression and multinomial logistic regression (Study II)

The distal outcomes in study II were dichotomous (for variables: *repeated testing for chlamydia during previous 12 months, current chlamydia test result*) and ordinal categorical with three response categories (for variables: *repeated lifetime testing for chlamydia, repeated lifetime chlamydia infection*) (**Figure *8B***). The relationship between LCs and these outcomes was assessed by applying an ordinal logistic regression model (210). The main assumption in this model is that the odds and the odds ratios are the same across categories of the outcome, hence proportional odds assumption. The null hypothesis of proportional odds can be assessed statistically via the Brant test (211). In case this assumption is violated, as it happened with one of our outcomes (*Lifetime chlamydia infection* in men), the multinomial (polytomous) logistic regression model can be used instead (212). The latter model allows varying relationship between exposure and categories of the outcome. Both models yielded ORs.

5.3.4 Time series negative binomial regression (Study III)

Time series are sequences of data points (disease, events) ordered in time, usually equally spaced in time. The main feature of time series is that observations close in time tend to be correlated with each other, which should be accounted for in the analysis (213). In study III, we investigated time series of reported incident chlamydia cases with monthly interval, and yearly interval when adjusted for the testing. Several models are applied to study time series, decomposition method and autoregressive integrated moving average (ARIMA) models are among the most popular ones in biomedical research (214). We chose a decomposition method, which extracts the underlying pattern from the time series: trend and seasonality. The trend characterises the long-term changes, and seasonality characterises cyclic changes within time: seasons of the year, holiday breaks etc.

Within the decomposition approach, we fit negative binomial regression models for chlamydia notification counts with corresponding population counts as the denominator. As described above, due to overdispersion of count data (incident chlamydia cases), the negative binomial regression model was a better choice than Poisson model (215). Negative binomial regression is based on the assumption of a Poisson-like distribution and handles overdispersion. Our models included an overall non-linear trend, overlaid with monthly effects to capture seasonal variations in incidence, and were adjusted for autocorrelation, i.e. correlation between numbers of cases from month to month. We modelled trends as restricted cubic splines (216) to capture non-linear features of the data in 1992–2004 and as a linear trend during period 2009–2018. Initially, seasonality was modelled with sine and cosine function, but due to asymmetrical behaviour of the cyclic component in our data we had to use another solution. Therefore, seasonality was modelled as fixed monthly effects averaging to zero over a full year. The models were fitted via maximum likelihood. We expressed the trend as monthly incidence rates (IRs, notified cases per 100 000 population) and seasonality as incidence rate ratios (IRRs), calculated as the ratio of each monthly IR relative to the average annual chlamydia IR for the corresponding year. Similarly, we fitted negative binomial regression models for the annual chlamydia IRs with population as an offset and adjusted for individual counties as fixed effects. Due to yearly aggregation of the data, we were unable to study seasonality in this model. Therefore, we estimated only annual trends of chlamydia IRs adjusted for the testing.

5.4 MATHEMATICAL MODELLING (STUDY IV)

The aim of the mathematical models is to describe transmission dynamic of the disease, where demographic, behavioural and biological factors play a role (217). The models are used for either prediction or understanding, and usually are simplifications of the complexity of the interrelation between pathogen and host. In the field of modelling infectious disease, various transmission models have been developed using classification of populations into susceptible (S), infected (I) and recovered (R) (218), a SIR model. These population subgroups (S, I, R) are referred in the model as compartments, which also gave a name to the compartmental

model. However, due to short-term complete immunity for chlamydia infection (219), recovered (R) are often disregarded from the model, resulting in a SIS (Susceptible-Infected-Susceptible) model. Individuals move between compartments with a certain rate, which is described by mathematical equations, which allows to estimate unknown parameters.

In study IV, we extended a deterministic compartmental SIS transmission model without demographic processes (birth and death) (114). The model is based on the surveillance data (chlamydia cases and testing), health care-seeking behaviour and chlamydia infection natural history. The main attribute of the deterministic model is that the model will generate the same outcome as compared to the stochastic model. In the model, *uninfected* (U) individuals become either *symptomatic* (S) or *asymptomatic* (A) at a certain rate (***Figure 9***). Symptomatic individuals seek health care where they are diagnosed and treated (at a *rate of diagnosis*), and then return to *uninfected* status. Asymptomatic infections are detected through screening programmes (at a *rate of screening*) or due to spontaneous clearance of chlamydia infection and return back to uninfected. Both S and A can also be identified by partner notification (PN), a parameter that we introduced in the model and allowed different rates depending on symptom status. The model assumes that the asymptomatic are tested with the same probability regardless of the risk behaviour. We calculated the *testing coverage rate*, the *symptomatic diagnosis rate*, and the *asymptomatic diagnosis rate* for sexually active population by sex and age groups. The model also takes into consideration the *probability of true positive* and *false positive laboratory test results*. Using the compartment model, we can describe the disease dynamics with a set of differential equations (Online Supplement Part 1 of Paper IV) and solve unknown parameters (Table 1 in Paper IV). We assumed that the model is in steady state, which implies that the number of uninfected, symptomatic and asymptomatic individuals does not change with time, i.e. per year.

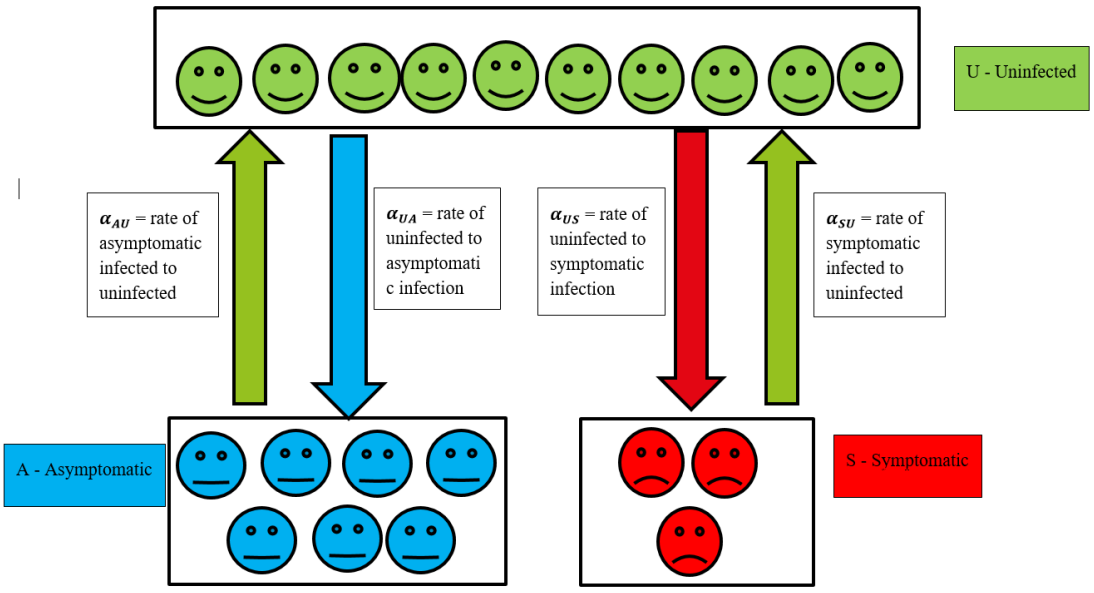


Figure 9. Schematic depiction of the model (U – uninfected individuals, A – asymptomatic chlamydia cases, S – symptomatic chlamydia cases).

We sampled each of the parameters 10 000 times from the appropriate assumed distributions (Table 2 in Paper IV), and assigned distribution to the reported data to obtain outcome estimates with uncertainty, 95% credibility intervals (CrI). We performed sensitivity analyses by testing one parameter at a time to evaluate how much each parameter has affected the outcomes. We varied the following parameters: the symptomatic testing rate, the asymptomatic testing rate, the probability of a false positive test, the probability of a true positive test, and the fraction of diagnosed cases that are asymptomatic, while using sampled distributions of size 10 000 for the remaining parameters.

5.5 ETHICAL ASPECTS

In studies I and II, we utilized data from questionnaires and clinical test for chlamydia collected at an STI clinic in Stockholm. All study participants were informed about the study aims and procedures, and were provided with written informed consent. In order to link the questionnaire and a clinical result, a study number was assigned to each study participant. In addition to the consent form, a contact form with study participants PIN and address details was generated for the follow-up invitation. The key for linkage of PINs and study numbers was kept separately in a secure file. All paper questionnaires were kept in locked storage. All individuals tested positive for *Chlamydia trachomatis* received treatment free of charge, and underwent partner notification according to the guidelines at the time. In addition, these individuals were offered test of cure approximately 4 weeks after being tested positive for chlamydia. According to the Biobanks in Medical Care Act (220), all clinical specimens were destroyed after the end of the study. For studies I and II, ethical approval was obtained from Regional Ethics Review Board in Stockholm (reference number: 2007/933-31/4). A supplementary application to Regional Ethics Review Board in Stockholm (reference number: 2011/313-32) was granted regarding change of research principal investigator.

In study III, we extracted data on chlamydia cases and number of tests from the national register for infectious diseases SmiNet-2, aggregated by county type and year. Aggregation level did not allow identifying individuals. However, we still applied for and received ethical approval for this study from the Regional Ethics Review Board in Stockholm (reference number: 2012/1710-31/3).

In Study IV, we used secondary data in aggregated format (age groups and sex), which did not allow identifying individuals. Additionally, we used parameters for the model from the published references. Therefore, ethical review was not required.

6 MAIN FINDINGS

6.1 STUDIES I AND II

6.1.1 Characteristics of the study participants

During the recruitment period, 2 814 individuals visiting an STI clinic agreed to take part in the study (recruitment rate: 53.7% (2 814/5 244)). Out of 2 814 respondents, 1 378 (49%) were women and 67.3% were single. The mean age was 27.4 years (27.0 for women and 27.8 for men). The median number of sexual partners during the previous 12 months was four partners for both sexes. The median number of casual sexual partners for women was two, and for men was three during the previous 12 months as reported by 74% of respondents. Of the study participants, 30% never or seldom used condoms with new or casual partners. During the past 6 months, 90% of participants used alcohol and 10% used drugs before having sex. A considerable proportion of the study participants reported lifetime testing for chlamydia (82%, 2 310/2 814), and 60% reported lifetime testing for HIV.

6.1.2 Factors associated with chlamydia infection

In this cohort of visitors to an STI clinic, 303 (10.7%) tested positive for *Chlamydia trachomatis*, with a higher positivity rate observed among men (12.6%) than in women (8.9%). We identified the following independent factors associated with a statistically significant increased risk for chlamydia: being 20–24 years old, being tested due to partner notification; symptoms as reason for testing; reporting 6–10 sexual partners during the previous 12 months; reporting last sexual contact as "petting, vaginal, oral, and anal sex"; consuming alcohol before having sex; as well as presenting symptoms (***Table 3***). We also identified a statistically significant strong protective effect of having been tested for chlamydia during the past 12 months compared with not being tested (***Table 3***).

Table 3. Factors associated with *Chlamydia trachomatis* diagnosis

Covariate	Adjusted Risk Ratio (95% Confidence Interval)	P-value*
Gender		
- Women	1.15 (0.84 - 1.56)	0.382
- Men	1.00	
Age group		
- 20-24	2.10 (1.21 - 3.65)	0.008
- 25-29	1.57 (0.91 - 2.72)	0.105
- 30-34	1.47 (0.81 - 2.66)	0.206
- 35-40	1.00	
Reason for current chlamydia testing		
- Casual sex/ check-up	1.00	
- Contact with chlamydia case/PN	6.55 (4.77 - 8.98)	<0.001
- Symptoms	2.19 (1.48 - 3.24)	<0.001
Ct test (past 12 months)		
- No	1.00	
- Yes	0.72 (0.55 - 0.94)	0.014
- Don't remember	1.10 (0.65 - 1.87)	0.730
Number of sexual partners (past 12 months)		
- 0-2 partners	1.00	
- 3-5 partners	1.12 (0.81 - 1.55)	0.498
- 6-10 partner	1.53 (1.06 - 2.21)	0.023
- ≥11 partners	1.61 (0.94 - 2.76)	0.082
Time since the last sexual contact		
- Last 7 days	0.70 (0.48 - 1.01)	0.056
- 1-4 weeks	0.95 (0.66 - 1.37)	0.784
- 1-3 months	1.00	
- ≥4 months	1.47 (0.71 - 3.05)	0.301
- Don´t remember	1.37 (0.20 - 9.43)	0.751
Type of the last sexual contact		
- Vaginal, oral and petting	1.00	
- Vaginal	1.17 (0.86 - 1.61)	0.319
- Vaginal and oral	0.77 (0.51 - 1.17)	0.219
- Vaginal and petting	0.81 (0.47 - 1.40)	0.460
- Vaginal, oral, petting and anal	1.84 (1.09 - 3.10)	0.023
Alcohol use before having sex (past 6 months)		
- Yes	1.98 (1.10 - 3.57)	0.023
- No	1.00	
Men presenting symptoms at clinic visit**		
- Yes	2.09 (1.38 - 3.18)	0.001
- No	1.00	
Women presenting symptoms at clinic visit**		
- Yes	1.08 (0.71 - 1.65)	0.706
- No	1.00	

* P-value from Wald test; ** P=0.017 for the interaction term “Gender x Symptoms”

6.1.3 Latent classes by sex

In study II, we were able to study a pattern of behaviours in order to identify unobserved (hidden) latent classes (LCs) as in contrast to study I where we studied independent factors. Based on nine selected observed variables, we identified four LCs for men and three LCs for women with increasing gradient of sexual risk behaviour, with class 1 being the least risky behaviour. We interpreted, labelled and ordered the latent classes based on the item-response probabilities. Among the men, 8% (n= 110) fell into class 1, labelled "Mixed steady and non-steady partnerships, low substance use" (***Figure 10***). Thirty percent (n= 441) of the men fell into class 2, labelled "Steady partnership with/without concurrent partners". They were considered medium risk, with a low probability of not having a steady current relationship and a higher probability of many sexual partners with and without concurrent relationships. For the men, we could further separate LCs of high-risk behaviour: "Non-steady partnerships with many partners, condom users" (class 3, n = 601) and "Non-steady partnerships with many partners, condom non-users" (class 4, n= 284), comprising 42% and 20% of the men, respectively.

Amongst the women, 10% (n=134) fell into class 1 of low-risk sexual behaviour, labelled "Mixed steady and non-steady partnerships, low substance use" (***Figure 11***). **Figure *11*. Latent class conditional probabilities for women (N=1 378)**Thirty-two percent (n=441) of the women fell into the class 2, labelled "Steady partnership with/without concurrent partners" (medium risk). The largest class 3, containing 58% (n= 803) of the women, was labelled "Non-steady partnerships with many partners".

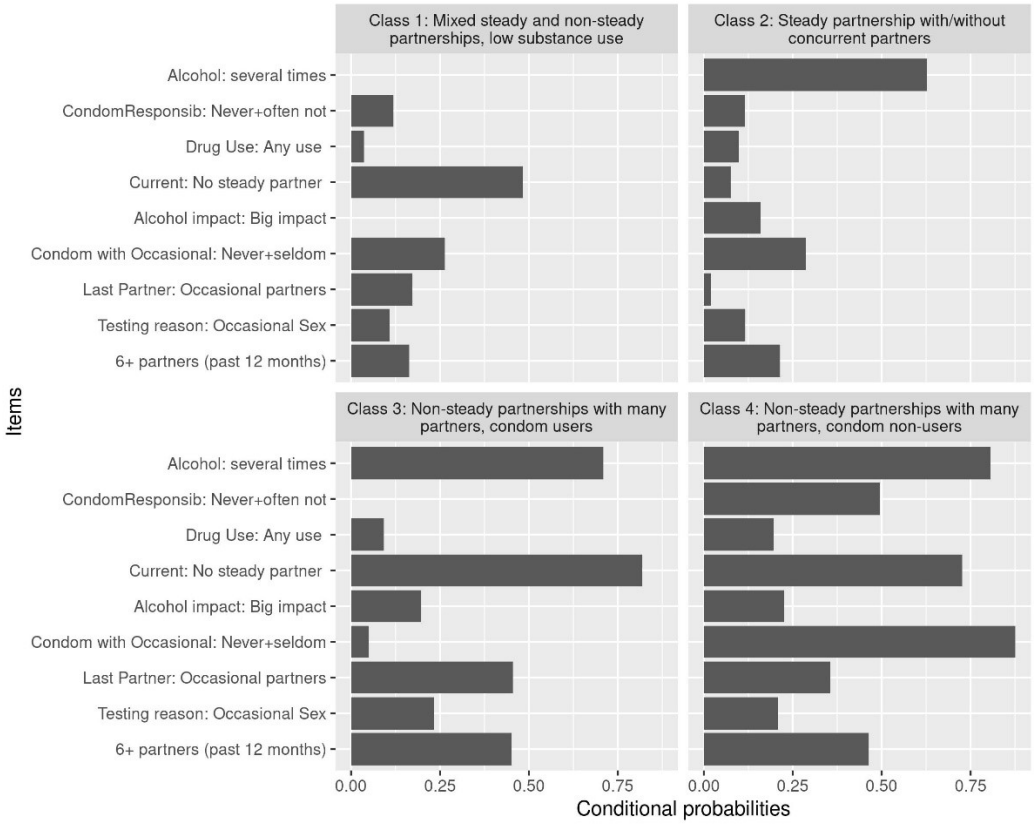


Figure 10. Latent class conditional probabilities for men (N=1 436).

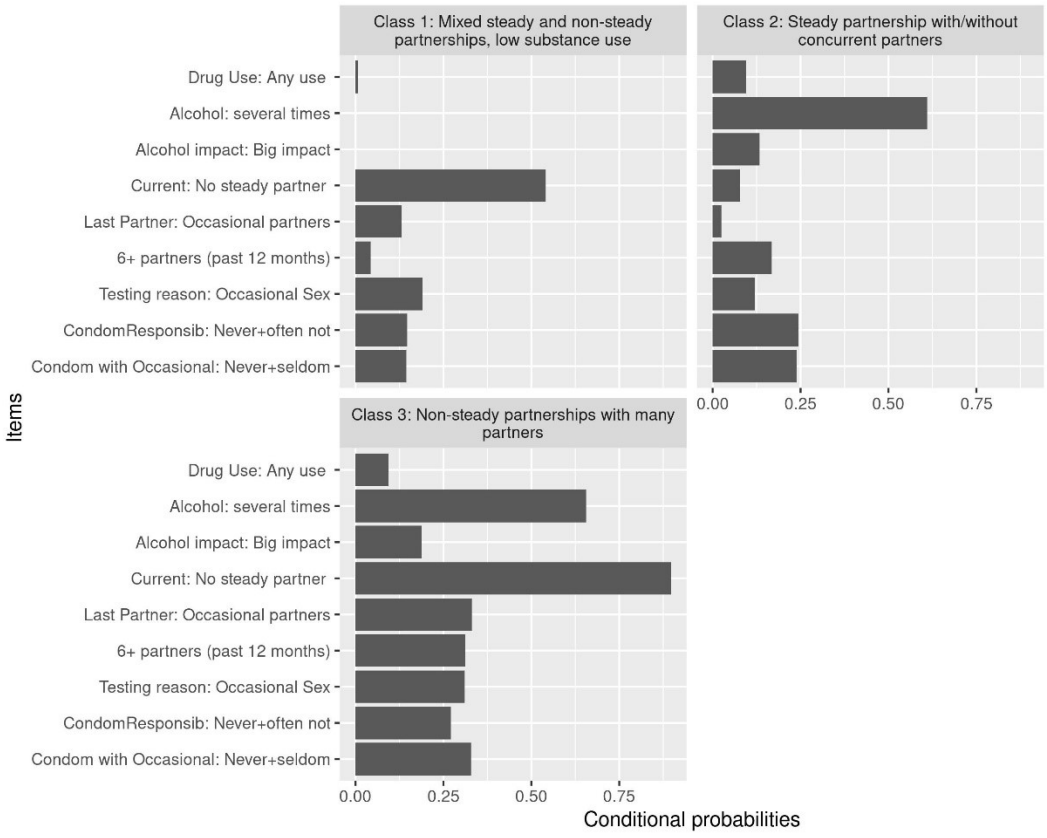


Figure 11. Latent class conditional probabilities for women (N=1 378).

6.1.4 Latent classes associated with repeated chlamydia testing by sex

In this analysis using proportional odds models, we identified that LCs with high-risk sexual behaviour (class 3 and 4) in men were statistically significantly associated with increased lifetime repeated testing for chlamydia (***Figure 12***). Among women, high-risk sexual behaviour (class 3) was also statistically significantly associated with increased odds of repeated lifetime testing. Additionally, in women, class 3 was also associated with repeated testing during the previous 12 months. Among men, we found only a borderline statistical association between the high-risk class 3 and repeated testing during the previous 12 months (***Figure 12***).

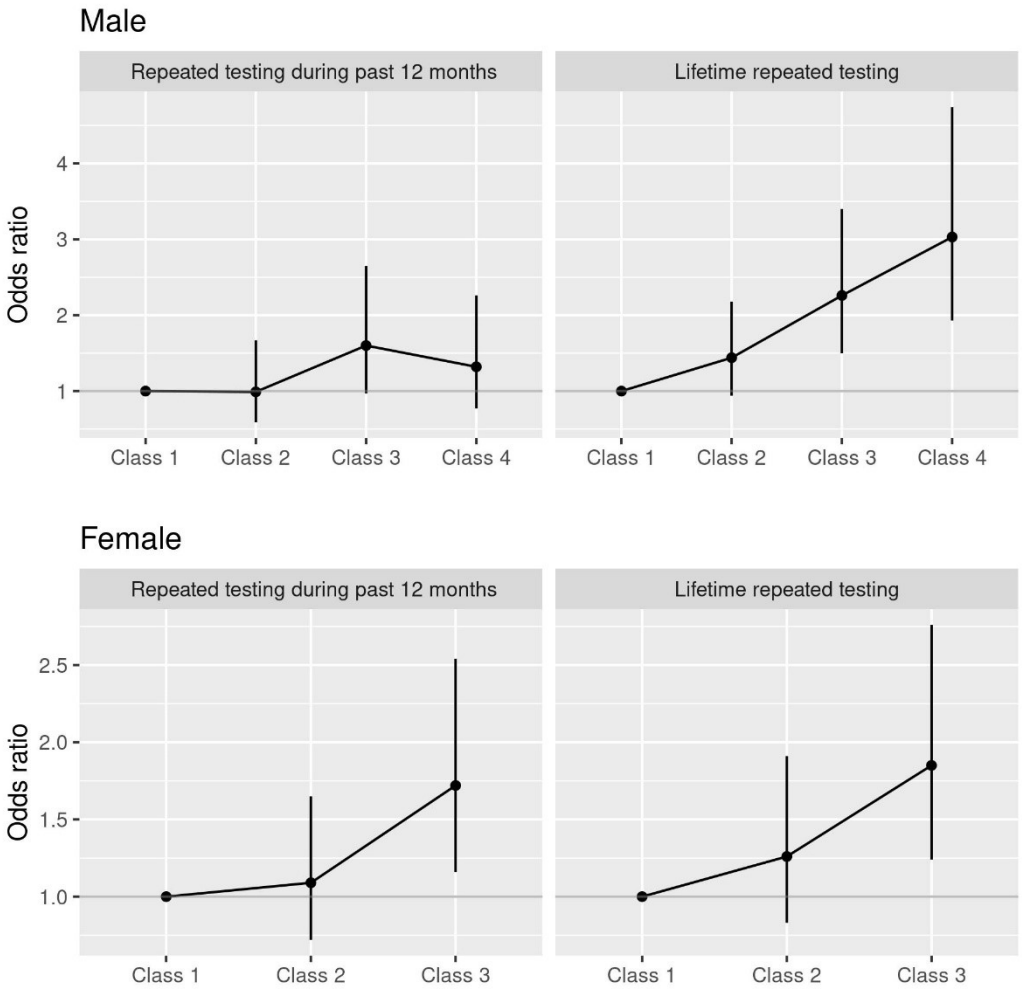


Figure 12. Association between latent class membership and repeated testing by sex, adjusted for age groups.

6.1.5 Latent classes associated with repeated chlamydia infection by sex

With a proportional odds model among men, we found that class 4 had statistically higher odds to test positive for current chlamydia infection than class 1 (***Figure 13***). Class 3 among men had borderline statistically significantly increased odds as well. However, for the outcome *Lifetime repeated chlamydia* (never, once, twice or more) in men proportional odds assumption was not met, and therefore a multinomial logistic regression model was applied, with no assumption on the odds between categories of the outcome. Thus, we identified that men in class 3 had 1.84 (95%CI: 1.03–3.26) higher odds of having a lifetime infection once compared to never having it (as compared to class 1). Respectively, class 4 had 2.54 (95%CI: 1.39–4.64) higher odds to have lifetime chlamydia once compared to never having it as compared to class 1 (***Figure 13***). The odds of having chlamydia infection twice or more compared to having had it once varied across LCs in men as well, though not statistically significant.

Among women, none of the associations were statistically significant for *Lifetime repeated chlamydia infection* or *current chlamydia infection* (***Figure 13***).

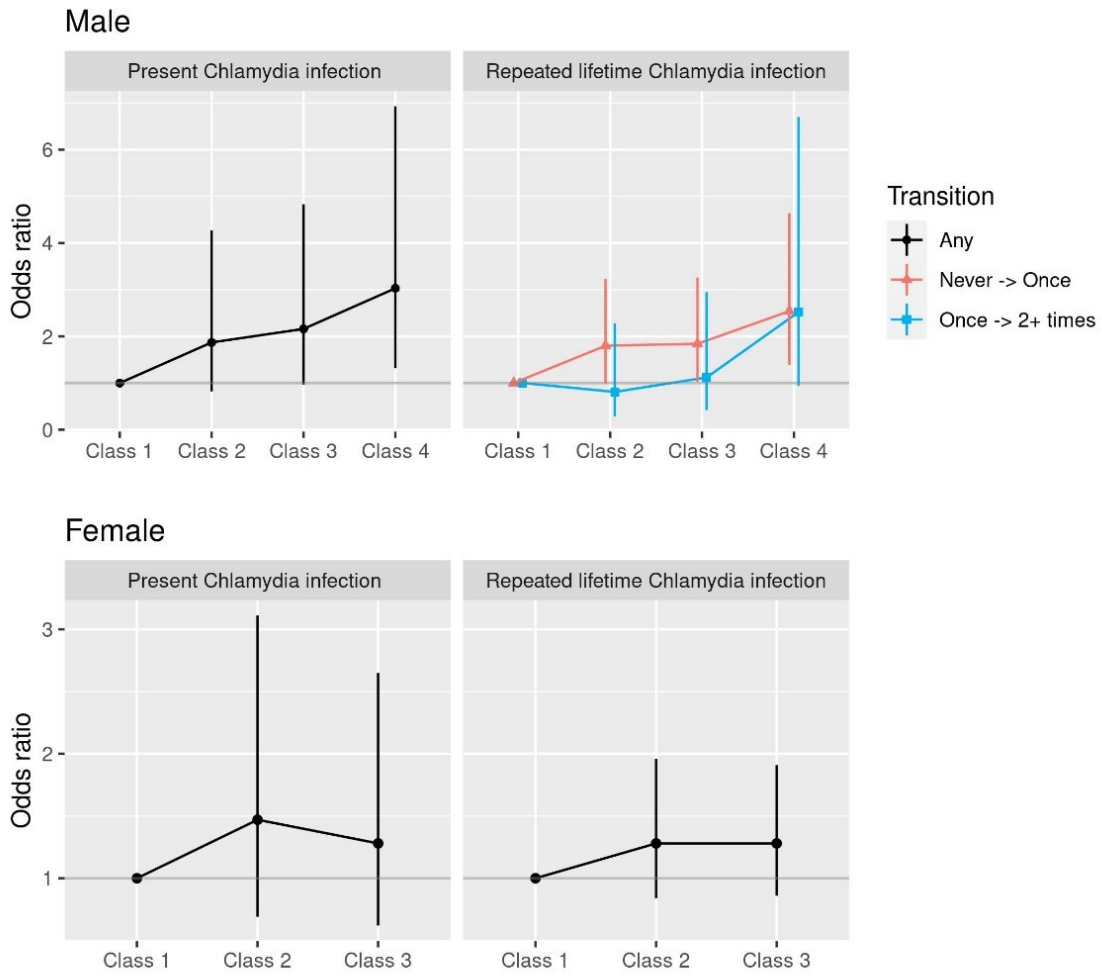


Figure 13. Association between latent class membership and Chlamydia infection by sex, adjusted for age groups

6.2 STUDY III

6.2.1 Characteristics of the study participants

In this analysis, we included 605 889 chlamydia cases reported during two periods: 1992–2004 and 2009–2018. During period 2009–2018, 361 330 cases (60% of included cases) were reported (***Figure 14***). Overall, among reported chlamydia cases, 58% were women and 85% were in the age group 15–29 years. For the analysis for two periods, we included 9 902 855 persons tested for chlamydia, of those 53% were reported during period 2009–2018. During the first period on average 77% of all tests were performed in women, as compared to 68% in the second period. The available data on the age groups among the tested population

during the period 2009–2018 revealed that on average 66% of the tested population were in the age group 15–29 years.

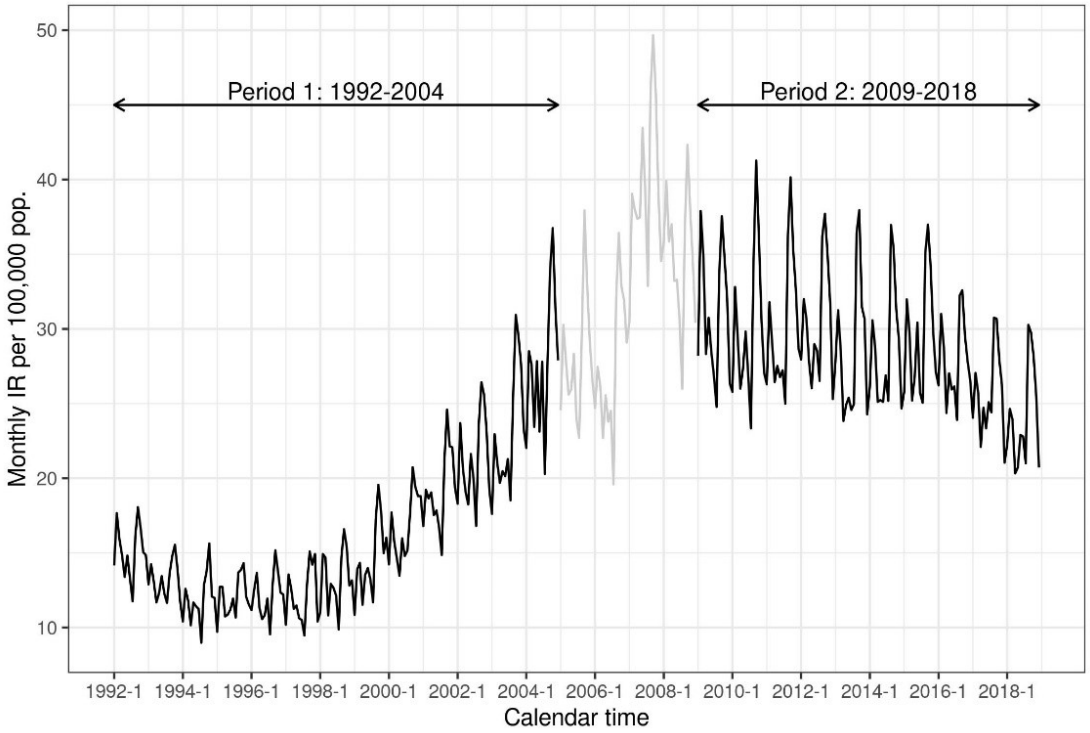


Figure 14. Reported monthly national incidence rates of chlamydia cases per 100 000 population in Sweden, 1992 to 2018. Excluded years (2005–2008) are highlighted in grey.

We calculated the proportion of the population (aged 15–64 years) tested annually, which was between 5.2% and 6.9% during 1992–2004 and between 7.2% and 8.5% during 2009–2018. During the first period, the proportion of the population tested annually was similar between able-to-detect (5.0–7.1%) and unable-to-detect (5.2–6.8%) groups. During the second period, the proportion of population tested was slightly higher, with 7.1–7.7% and 7.2–9.0% in each group, respectively.

6.2.2 Times series trends

We found that the national trend for chlamydia cases follows a U-shape during the period 1992–2004 (**Figure *15A***). The incidence declined from the start of the period, reaching the lowest IR in 1996, followed by a subsequent increase of 83.7% between 1996 and 2004. The second period (2009–2018) began with higher IRs level than at the end of the first period (**Figure *15B***). Initially a stable trend decreased slightly from 2015 and onward. Overall, the rates decreased by 19.7% from 2009 to 2018. The trends were statistically significantly different between the two periods (P<0.001).

We found similar trend patterns by groups of counties as was seen for the national trends (***Figure 15C-D***). Chlamydia IRs were higher in unable-to-detect counties from 1992 to 2001 (***Figure 15C***). After 1996, however, the trend in unable-to-detect counties increased at a slower rate (by 75%) than in the able-to-detect counties (by 159%) towards 2004, being statistically significantly different (P<0.001). Similarly, throughout the second period, the estimated IRs in the unable-to-detect group were higher compared to the rates in the able-to-detect group as well (***Figure 15D***). From 2009 to 2018, a decrease of 11.6% in the able-to-detect and of 23.5% in the unable-to-detect group was seen, with both trends almost converging towards the end of 2018.

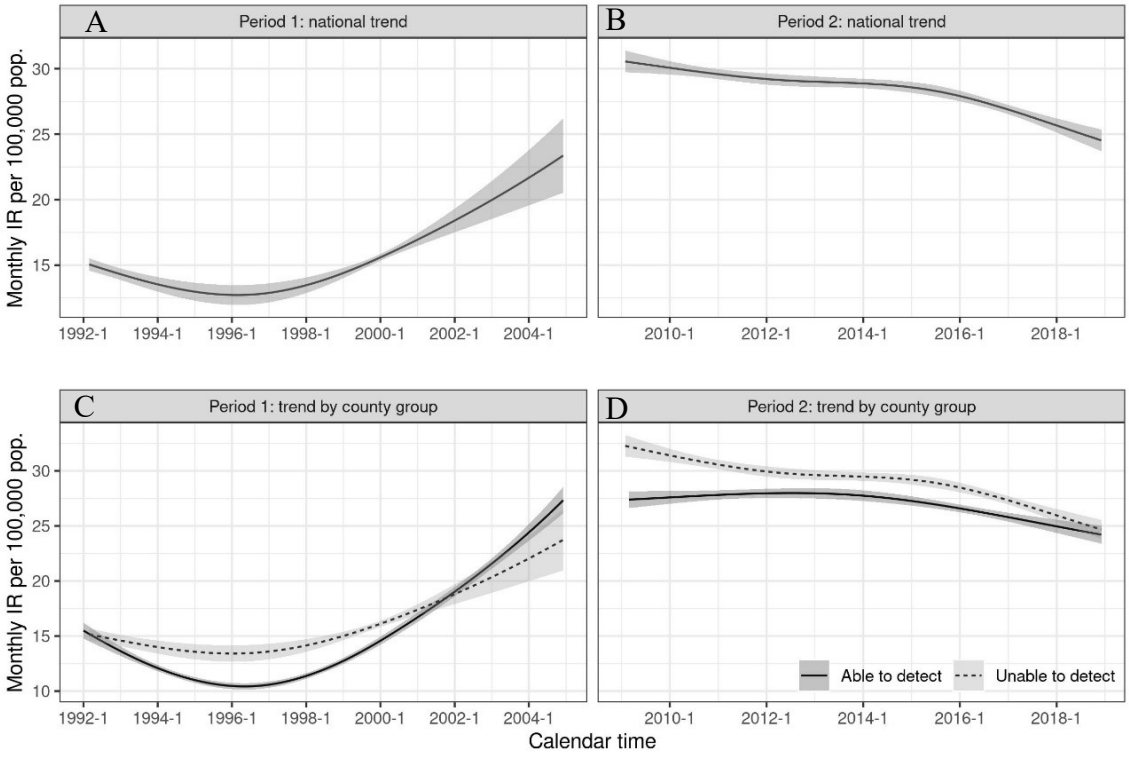


Figure 15. Estimated chlamydia trend (monthly IR per 100,000 population), 1992 to 2004 and 2009 to 2018, for the national chlamydia cases (A and B) and by group of counties (C and D). A and B, Solid black line represents national IRs; shaded area represents 95%CIs. C and D, Black solid line represents able-to-detect counties; black dashed line represents unable-to-detect counties. Shaded area represents 95% CIs.

6.2.3 Seasonality

The national within-year periodicity of chlamydia cases was similar in both periods: the highest IRs compared to the annual average of chlamydia IRs were seen in months 9–11 (autumn), and the lowest IR in month 8 (summer) and month 1 (mid-winter), with the autumn peak slightly shifted in the second period (***Figure 16***). Periodicity was statistically significantly different between periods (P<0.001).

Seasonality patterns were identical to the national model, with no statistically significant differences found between groups of counties within each period.

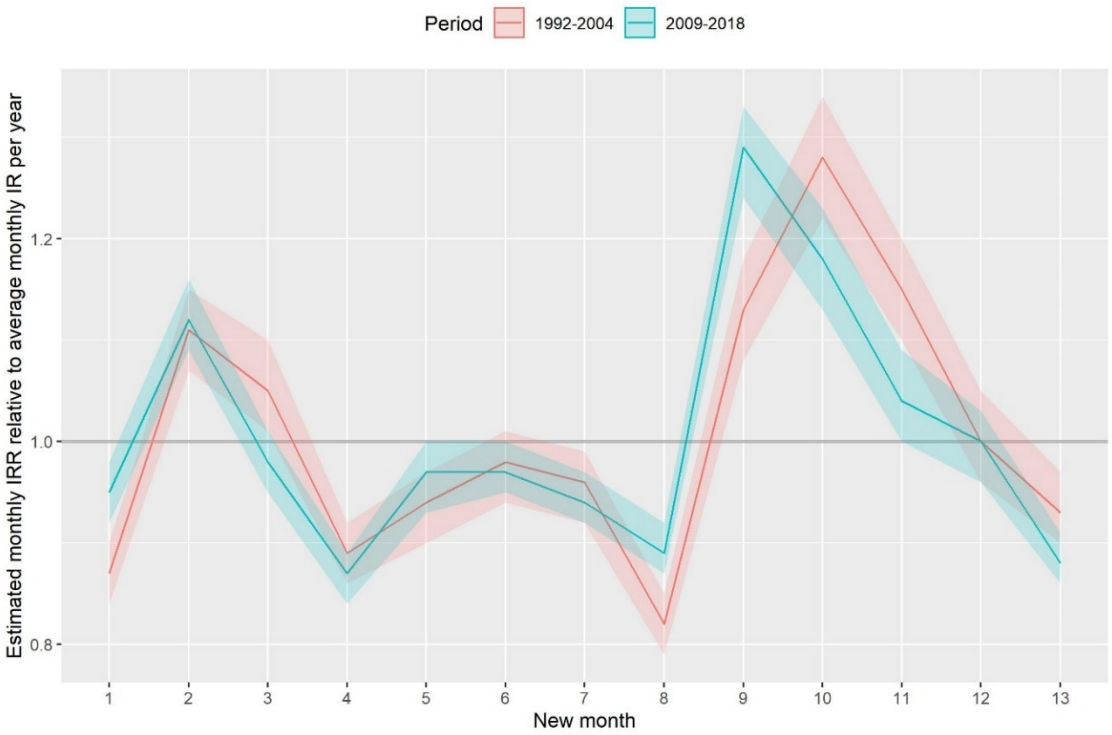


Figure 16. Estimated periodicity (monthly Incidence Rate Ratio) from fitted model for the national number of chlamydia cases in Sweden, 1992-2004 and 2009-2018.

6.2.4 Association between testing and chlamydia rates

Based on the annual data, we estimated that an increase in the proportion of the tested population (aged 15–64 years) was statistically significantly associated with an increase in chlamydia IRs in the first period. However, this effect declined over time (interaction term, $P<0.019$). Hence, for a one percent increase in the proportion of the population tested, we estimated an expected 7.0% (95%CI: 4.1%–9.5%) increase in annual chlamydia IRs in 1992, but the corresponding annual increase in chlamydia IRs in 2004 was only 2.3% (95%CI: 0.01%–4.9%) (***Figure 17A***). Similarly, the predicted effect of increased testing on estimated IRs diminished between 1992 and 2004 in each county group. In order to visualize this effect, we predicted chlamydia IRs from 1992 to 2004 for a hypothetical increased annual testing coverage by 1% for each year. The predicted counterfactual chlamydia IRs were higher throughout the period, although with a strongly diminishing increase in chlamydia IRs in the able-to-detect group towards 2004 (***Figure 17B***). For the period of 2009–2018, we did not find a statistical association between the proportion of the population tested and chlamydia IR, either at the national level or by group of counties.

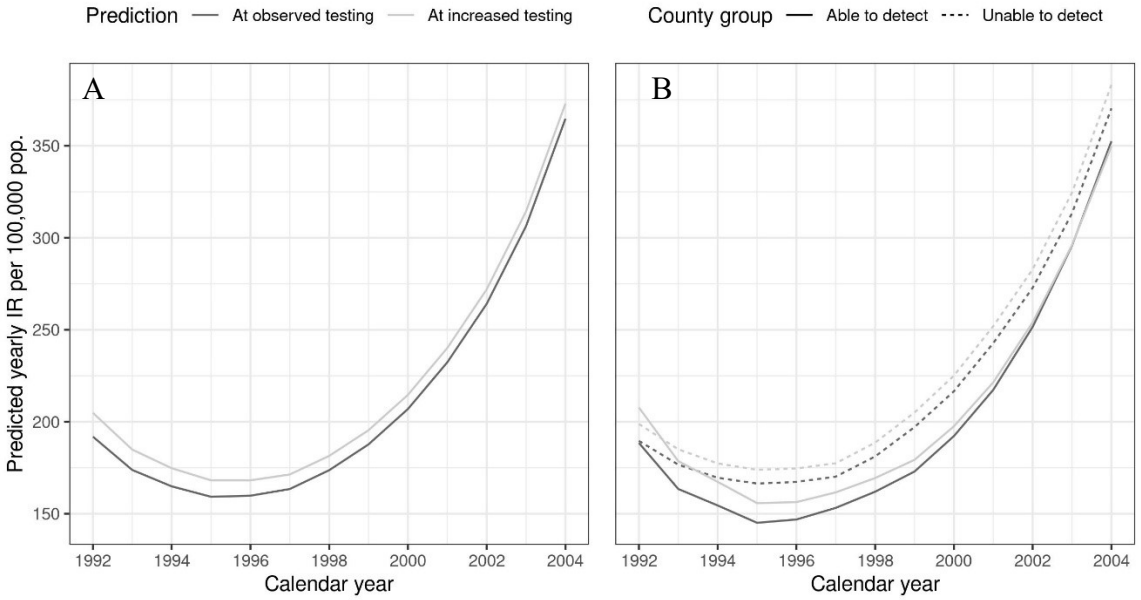


Figure 17. Estimated annual chlamydia incidence rates adjusted for proportion of population tested nationally (A) and by group of counties (B), 1992 to 2004.

Solid black lines represent estimated IRs from the model, grey solid lines represent counterfactual IRs with 1% increased proportion of tested populations (A).
Black solid lines represent able-to-detect counties estimated IRs from the model, grey solid lines represent counterfactual IRs with 1% increased proportion of tested populations in able-to-detect counties (B).
Black dashed lines represent unable-to-detect counties estimated IRs from the model, grey dashed lines represent counterfactual IRs with 1% increased proportion of tested populations in unable-to-detect counties (B).

6.3 STUDY IV

6.3.1 Estimated chlamydia prevalence

Our model estimated similar patterns of prevalence among women and men: an increase between 2009 and 2012, followed by a decrease in 2015 and 2018 (***Table 4***). Overall chlamydia prevalence in the age group 15–29 years was approximately 1.6 times higher in women compared to men in all years of the study.

Table 4. Estimated median chlamydia prevalence (in percent) in the age group 15–29 years among sexually active men and women in Sweden, 2009, 2012, 2015, 2018.

	Men		Women	
Year	Median prevalence	95% Credibility interval	Median prevalence	95% Credibility interval
2009	3.73	2.31–7.67	5.87	5.25–6.61
2012	3.93	2.27–8.33	6.14	5.45–6.93
2015	3.36	2.04–7.03	5.29	4.71–5.95
2018	2.33	1.53–4.55	3.62	3.22–4.06

Estimated chlamydia prevalence ranged between 1 and 7 % depending on year, age group and sex. The highest chlamydia prevalence was estimated for all years in age group 15–19 years, followed by the age group 20–24 and 25–29 years (***Figure 18***) with the exception of 2009 when the highest prevalence was estimated in men in age group 20–24 years instead. In general, we estimated the decline in prevalence between 2009 and 2018 with biggest decline seen in all age groups and both sexes between 2015 and 2018. The prevalence in 2015 in 15–19 year old men was 5.3% (95%CrI: 4.35%–6.29%) and dropped to 3.6% (95%CrI: 2.76%–4.39%) in 2018, a drop by 32.5%. Among 15–19 year old women respectively, the prevalence in 2015 was 5.7% (95%CrI: 5.25%–6.22%) and dropped to 3.7% (95%CrI: 3.38%–4.19%) in 2018, a drop by 34.6%. Similar percentage drops were estimated in the remaining age groups and sexes between 2015 and 2018, which resulted in the lowest prevalence estimates in 2018 (**Figure *18***).

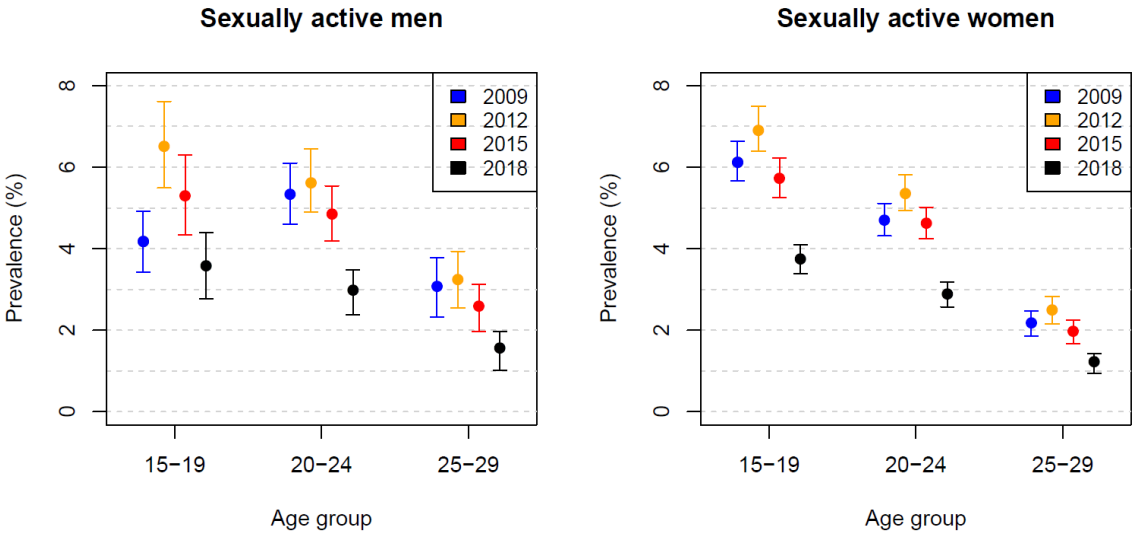


Figure 18. Estimated chlamydia prevalence among sexually active men and men by age groups in Sweden, 2009, 2012, 2015, 2018 (vertical bars represent credibility intervals).

6.3.2 Estimated chlamydia incidence

The estimated (true) incidence rates were consistently higher compared to the notified chlamydia incidence rates in the surveillance system: in the range of 1.6–2.2 times higher in men and 1.8-2.9 times higher in women in the population 15–29 years of age (**Figure *19***; note, that for the comparison with notified incidence rates, we recalculated true incidence rates with the whole population as denominator). This implies that every year we potentially under-diagnose between 26 829–56 103 chlamydia cases among women and between 16 626–29 647 chlamydia cases among men (estimated for 2009, 2012, 2015, and 2018).

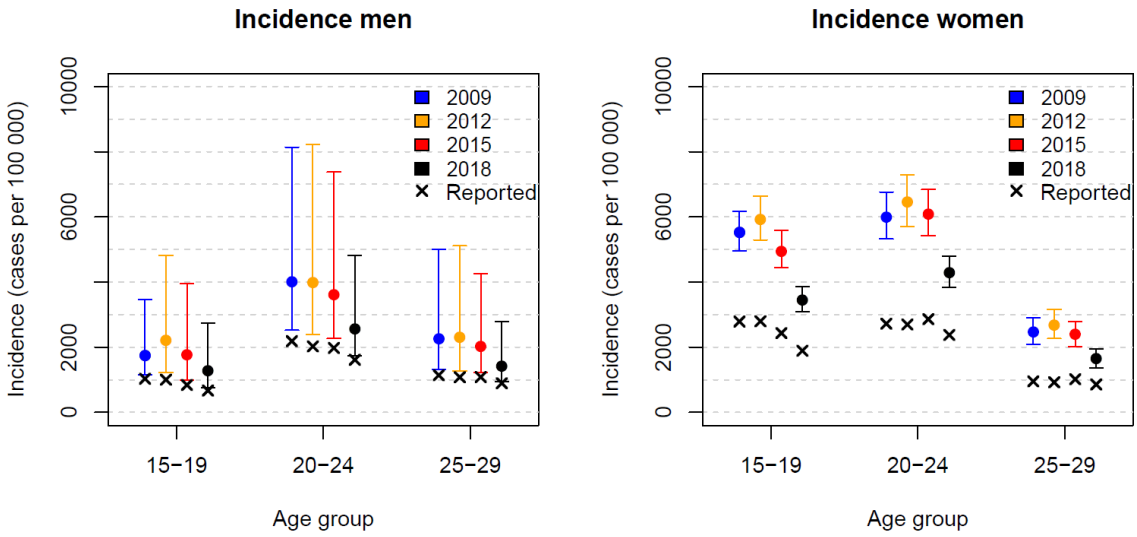


Figure 19. Estimated chlamydia incidence rates (dots with bars) and notified (crosses) chlamydia rates per sex and age groups in Sweden, 2009, 2012, 2015, and 2018 (denominator: total population, including not sexually active population). Vertical bars represent 95% credibility intervals.

6.3.3 Estimated proportion of asymptomatic chlamydia cases in the population

We estimated the median proportion of asymptomatic (including undiagnosed chlamydia cases) cases to be 83% for men and 88% for women in the general population aged 15-29 years. The lower bound of 95%CrI for the proportion of asymptomatic cases was 65% among men and 82% among women, while the upper bound reached 95% and 91%, respectively. In comparison, the median proportion of notified asymptomatic cases in the surveillance system was 70% for men and 76% for women. While the proportion of asymptomatic cases notified due to PN was 40% among women and 66% among men (151).

6.3.4 Sensitivity of analysis results

Sensitivity results revealed that prevalence decreased if the probability of a false positive test increased, since then the observed number of cases is partly explained by the higher false positives and the true prevalence is lower. Prevalence decreased if the probability of a true positive test increased, as tested individuals would be infectious a shorter time. The prevalence increased when the proportion of diagnosed cases that were asymptomatic increased. The prevalence was unaffected by the spontaneous clearance rate and by time to seeking treatment based on symptoms if the values were close to ours (18.7–20.0 days), however, slightly increased when longer than 6 months due to the fact that infected individuals stay infectious a longer time.

The incidence increased when there was an increase in the spontaneous clearance rate and increase in of the proportion of diagnosed cases that were asymptomatic. The incidence decreased when the probability of a false positive test decreased and the probability of a true positive test increased, since infected individuals are infectious a shorter time. The incidence was unaffected by the symptomatic treatment seeking rate.

The probability of an incident case to be asymptomatic decreased with an increased probability of a false positive test. This probability of an incident case to be asymptomatic increased the probability of a true positive test; and increased if the proportion of diagnosed cases that were asymptomatic increased.

7 DISCUSSION

In the studies included in this thesis, we identified risk factors for chlamydia infection and risk behaviour patterns associated with repeated testing. We also concluded that chlamydia notification rates were driven by testing during 1992-2004, but not during 2009-2018. Finally, we estimated a decline in chlamydia prevalence between 2009 and 2018, which supported our hypothesis on the reason for the declining observed chlamydia rates. Below I discuss our findings and acknowledge the strengths and limitations of our studies.

7.1 INTERPRETATION AND IMPLICATIONS

7.1.1 RISK FACTORS

In studies I-II, we used data from a large cohort study from an urban STI clinic. We identified and updated the knowledge on independent risk factors associated with chlamydia diagnosis in study I. The identified independent risk factors, such as being young, reporting more than 6 sexual partners during the previous 12 months, reporting sexual activity involving oral/vaginal/anal contact, consuming alcohol before sex, testing due to partner notification, and having symptoms were consistent with risk factors reported in previous studies within and outside Sweden (77-79, 83, 84, 221). Although we could not confirm the association between condom non-use with casual partners and chlamydia infection, other studies have reported that consistent use of (male) condoms are effective in preventing chlamydia (222, 223) and other STIs (224). Previously identified risk factors for chlamydia were utilized elsewhere to develop algorithms to support selective screening (225-227). The presented prediction models provided prognostic accuracy in separating test positive and test negative individuals, suggesting it could facilitate selective chlamydia screening even at the population level (93). However, several other studies reported failure to identify accurate screening algorithms with suggested underlying reasons (228, 229), which need further investigation and evaluation.

In study II, based on sexual behaviour and substance use, we found distinct latent classes consisting of patterns ranging from low- to high-risk behaviour. Among men with high-risk behaviour, we could specifically distinguish between condom users and non-users. In high-risk behaviour LC in women, however, only one class was identified, with 33% who never or seldom used condoms. Previous studies have reported that even when respondents consider it important to use condoms and intend to use them, the actual use varies by type of partner and type of sexual contact (230-234). In Sweden, hormonal contraceptives have been the first choice of contraception over the past decades as respondents stated more concern regarding unwanted pregnancy than prevention of STIs, and thus condom use has not been a priority (122, 235, 236). The latest data also suggested that condom use has decreased over time between 2007 and 2013 among 15-24 year old individuals in Sweden (236).

Additionally, we found a high prevalence of alcohol consumption in moderate- and high-risk behaviour LCs. Previous studies reported that alcohol consumption in close proximity to

the sexual encounter could contribute to sexual risk-taking due to poor judgement on the choice of casual sexual partner, multiple sexual partners, increased probability of condomless sex, and therefore increase in the risk of chlamydia acquisition (237-240). Furthermore, in our high-risk classes, we identified combined substance use (alcohol and drugs), which is also reported previously to be linked to increased sexual risk-taking behaviour (90, 237). Sexual risk-taking is a complex topic, where other researchers have studied personality characteristics (241, 242) besides the variables included in our latent class membership (such as sexual behaviour and substance use), which can further identify subgroups with greater need for prevention.

The results from study II showed that women and men in high-risk behaviour LCs had higher odds to test repeatedly for chlamydia during their lifetime. Furthermore, women in the high-risk behaviour class had higher odds to test repeatedly for chlamydia during the previous 12 months, but no such association was found in men. This suggests that current efforts to promote testing are adopted by individuals in high-risk behaviour classes, both women and men (189). Generally, men are under-represented among the chlamydia tested population, thus in Sweden, only 30% of all tests are performed in men (32). Of all tests over 20% are from internet-based services, where the number of tests in men, however, increased by 71% between 2013 and 2017 (156). Adolescents and young adults are at higher risk for chlamydia (due to multiple sexual partnerships and casual sexual contacts) and therefore, re-testing of chlamydia positive individuals is recommended with varying intervals (3-12 months) (33, 165). Evidence suggests that repeated testing after the initial infection is beneficial, since a high proportion of repeat chlamydia infections can be found (102, 104, 243-245), and therefore further transmission can be prevented. Thus, repeated testing for chlamydia is commonly reported elsewhere (246-248).

In study II, we found that men in the high-risk behaviour class 4 were more likely to test positive for chlamydia twice or more times during their lifetime, however with borderline significance. This finding may indicate that no change in sexual behaviour occurred for these repeatedly tested men over time. Similar findings were reported in previous studies where LCs of high risk-takers were more likely to contract STIs (249-252). As described above, condom use with casual partners in high-risk behaviour classes was low or moderately low; indicating that promotion of condom use should be reinforced. However, effective communication to reach the population at risk with safe sex messages is still under research (253, 254).

7.1.2 INCIDENCE TRENDS AND PREVALENCE

In studies III and IV, we utilized data on notified chlamydia cases and testing for chlamydia. We found that chlamydia notification rates at the national level and by county groups (able- and unable-to-detect nvCT) during the period 1992–2004 had a U-shaped trend, with several proposed reasons for such a trend. Initially, the decrease in the late 1980s and early 1990s was thought to be attributable to fear for the emergence of HIV infection and following prevention campaigns (40, 255, 256). This affected also trends of other STIs in Sweden and

elsewhere: gonorrhoea rates declined by 40–70% in most western EU countries, as did syphilis rates, a decline by 10–60% (257). After a relatively short plateau, chlamydia and other STIs (gonorrhoea and syphilis) notification rates started to rise from the mid-1990s and onward. Possible reasons for the rise elsewhere and in Sweden were suggested as follows: the introduction of more sensitive diagnostic methods (NAAT), possibly increased chlamydia prevalence in the population, as well as a switch to chlamydia high-prevalence target groups for testing (44-47). The introduction of antiretroviral therapy for HIV was also considered to contribute to that rise (258), as sexual risk-taking was reported to increase as well, such as a decline in condom use (259). Another suggested reason for the rising trends is a hypothesis of arrested immunity (260). The theory suggests that treatment for chlamydia interrupts the development of natural immunity, therefore making individuals susceptible to re-infection. This was supported by the findings in studies elsewhere, reporting that an increase in the chlamydia rates is actually seen due to repeated infections (261, 262).

The discovery of a new variant of chlamydia (nvCT) in 2006 (52) affected notified chlamydia rates in Sweden primarily because of false-negative test results (56, 263). In addition, our analysis by county groups (able- and unable-to-detect nvCT) showed that the trends of chlamydia notification rates differed already by county type from 1992 through 2004 (pre-nvCT). The possible differences may lie in varying testing coverage, which we describe below. Additionally, possible differences in the organization of partner notification process (centralized versus decentralized) (181, 183), varying success in reaching identified partners of index cases (264), and varying investments in primary and secondary prevention (265) could explain these differences between counties. Following the U-shape pattern as it was seen at the national level, trends have increased at the different rate already since 1998 depending on county type. The emerged nvCT cannot fully explain the differences in the observed increase because the estimated emergence of nvCT was in 2002-2003, with a gradual spread (54). The role that nvCT had on the chlamydia trends after its discovery was the reinforcement of control measures, with intensified testing efforts and partner notification. In the second analysis period (2009-2018), chlamydia rates declined consistently in both types of counties, although again at a different pace. The proportion of nvCT among all cases dropped to 5% in both types of counties towards 2015 (57), as compared to as high as 64% in unable-to-detect and as high as 19% in able-to-detect counties in 2007 (55). With nvCT being under control and unable to affect chlamydia trends in the latter study period, we suggest that chlamydia notification rates could have been affected by testing volumes which we investigated further in our study.

Moreover, in study IV, we estimated true incidence rates to be 2 to 3 times higher than notified rates between 2009 and 2018, which is anticipated, since chlamydia infection is often asymptomatic (as was also estimated in our study), and therefore not all cases are identified (266). Due to slow natural clearance (69, 74, 76), if not tested and treated, asymptomatic chlamydia-infected individuals can contribute to the ongoing transmission and fuel the epidemic. In fact, it was reported that antibodies for past chlamydia infection could be found in a third of 16–24 year old individuals who never previously had been tested for chlamydia (171), which in turn, can potentially contribute to the development of sequelae (2, 3, 267).

Therefore effective approaches to screening (179) should be researched further, especially in light of vague evidence of the effectiveness of opportunistic testing (opportunistic screening) (268), which is in place in Sweden. Furthermore, special attention should be dedicated to men's screening since their test-seeking behaviour is less successful (119, 120, 156, 247).

In study III, we studied the role of testing as one of the possible contributors to the change in the national notification trends over time, and possible underlying reasons for chlamydia rates being different between two types of counties (able- and unable-to-detect nvCT at the time). We found that increasing testing levels were associated with an increase in chlamydia notification rates during 1992–2004 but not during 2009–2018. Previous studies reported a strong relationship between increasing testing rates and increasing notified chlamydia rates (42, 46, 269-271), including repeat testing after the previous positive test (100, 248). Since the mid-1990s, many efforts were made to expand opportunistic testing in Sweden by offering testing at outpatient clinics (including drop-in), and since the early 2000s self-sampling at home and sample analysis at the laboratory, at no cost (156, 272). In the first period, increasing notified chlamydia IRs were dependent on the increasing testing coverage, suggesting likely underestimation of the true incidence rates, and more likely reflecting improvement in the diagnostic, reporting, and testing coverage (273). A similar influence of time-varying biases on the notified case rates was reported elsewhere (51). In the second period, however, our results suggest that notified chlamydia IRs is no longer driven by the testing volumes in the population targeted for testing as it was in the first period. Thus, notified chlamydia IRs more reliably mirror true incidence rates in the second period and possibly indicate a true decline in chlamydia incidence rates, which we investigated in study IV.

We investigated several possible reasons for the observed decline in chlamydia notification rates during 2009–2018. First, we looked at a possible change in the profile of the tested population (demographics) as was reported elsewhere (270). Available limited data suggests that no major changes occurred in terms of the distribution of the tested population by sex and age groups: two-thirds of women and the same proportion of individuals aged 15–29 years were among the tested population annually. Subsequently, in study IV, we investigated whether the decline in chlamydia prevalence could be the reason for the decreasing notified chlamydia rates seen in Sweden during 2009–2018. Indeed, we estimated a decline in chlamydia prevalence in 15–29 year old individuals during that period, with the biggest drop (at least 30%) in prevalence seen between 2015 and 2018 in all age groups and both sexes. When the duration of the infection is reduced, the prevalence is being reduced as well, and as a result, the transmission of the infection is abridged, which indicates the success of the screening program (274). From the point of the hypothesis of the dynamic topology of STIs epidemics (66), we suggest that the chlamydia epidemic evolved through time and potentially reached the decline phase because of prevention and control measures, by faster identification of the infected individuals, treatment, and PN, thus reducing the duration of the infection. Estimated prevalence by age group and sex in our study were consistent with population-based estimates obtained through various methods in the UK (84), the Netherlands (116), Norway (79), France (115), and other European and high-income countries (1, 117, 118). However,

prevalence is closely related to the population being tested, and consequently depends on sexual networks and core groups coverage (105, 273), which we did not consider in our modelling study.

Partner notification is an important component of chlamydia control strategy in Sweden, and therefore we included it in the model in study IV within screening rate for both chlamydia positive and negative individuals. In our surveillance data, most infected asymptomatic men were identified through PN (on average, 70% during the study years), while only 16% were identified through the screening (average during the study years) (151). This indicates that these men would be missed if PN would not include them. In the surveillance system, on average 40% of asymptomatic women are identified through PN and 30% via screening (average during the study years) (151). However, in our study we could not estimate the individual contribution of control measures on the prevalence. Previous modelling studies suggest that increased effectiveness of PN could be as effective in reducing chlamydia prevalence as increasing screening coverage (178, 179, 275). Partner notification is also effective in reducing the chlamydia burden at the population level and decreasing re-infection at the individual level (178, 276, 277). By including information on PN in our model in the screening rate for both chlamydia positive and negative individuals, we achieved a more realistic model for Swedish conditions and improved the precision of outcome estimates.

Generally, understanding the effectiveness of screening on chlamydia prevalence comes from modelling studies (178, 275, 278-280), Meanwhile, randomised and non-randomised controlled trials indicate uncertainty regarding the effect of screening on the prevalence, while moderate-quality evidence exists regarding the effect of chlamydia screening on the incidence of PID and chlamydia reinfection (159). Furthermore, to our knowledge, opportunistic testing (opportunistic screening) has never been evaluated elsewhere, while several countries continued to scale up their testing for chlamydia (154). Besides the potential benefits of the screening, some concerns were also reported. It was argued that screening programs for chlamydia in a low-risk population could be hampered because the majority of tested individuals would be tested negative and therefore might change their sexual behaviour towards riskier (281). This was supported in several studies reporting that repeated testing may facilitate only short-term change in high-risk behaviour if an individual was tested positive for chlamydia (282, 283), and those tested negative may change towards more risky behaviour (281) (284), suggesting unintended consequences of the testing (285-287). Additionally, the arrested immunity hypothesis suggests that individuals are susceptible to chlamydia after treatment due to interrupted development of natural immunity (260). This puts individuals at risk for repeated infections, suggesting potential harm of the screening, especially in the countries where partner notification (PN) is not part of the case management (136). It has to be said that the potential harm of screening for chlamydia has never been evaluated either (159).

7.2 STRENGTHS

A core strength of studies I and II is their large sample size: we were able to recruit almost 3 000 individuals, who answered the questionnaire and provided a biological sample. To promote unbiased answers, the questionnaire was provided to the study participants before the sampling for chlamydia, so that the study participants were unaware of their chlamydia status when answering, thus, minimizing the social desirability bias. Even though the overall missingness in the questionnaire data was comparatively low, we still choose to use a multiple imputation approach in study I in order to increase the power and provide valid inference (206). Additionally, the laboratory verification of the chlamydia status (as opposed to self-reported status) ensured unbiased ascertainment of the outcome.

Study II is, to our knowledge, the first study to investigate the association between sexual risk behaviour patterns and repeated testing. The latent class analysis approach allowed us to identify a range of behavioural patterns as well as sub-groups of individuals at higher risk for repeated chlamydia testing. Thus, providing information for future prevention and control measures.

A key strength of studies III and IV is the use of high-quality national surveillance data, which has been formally evaluated according to the guidelines of the Centers for Disease Control and Prevention (288). The Swedish chlamydia surveillance system that was used to generate this data has previously been described as useful, timely, and accurate (147). Another strength was the length of follow-up that allowed the use of a 23-years long series of data on chlamydia cases and testing, comprising a total of 606 000 cases and nearly 9.9 million tests for chlamydia.

A specific strength of study IV was the availability of partner notification information, allowing us to adjust our model to the Swedish context. The addition of the PN parameter was vital, as the distribution of asymptomatic chlamydia cases in Sweden differs substantially by testing reason (partner notification vs. screening). Our model also distinguishes between testing in the absence or presence of symptoms, by assigning different rates. Additionally, we also extended the original model by introducing different transition rates for those who tested negative and positive for chlamydia (114). We believe that this refinement of the model also improved the precision of our estimates.

7.3 METHODOLOGICAL CHALLENGES

After a study is conducted, it is crucial to assess to what degree the results are an accurate representation of the underlying truth, and whether and to what degree chance, potential biases, or the study design may have played a role. Below I discuss the methodological challenges encountered during the entire research project.

7.3.1 Study design

7.3.1.1 Cohort study

Cohort design is a type of observational study design where a cohort of individuals characterized by some common characteristic (e.g. individuals born in a particular year) is followed-up to an observed outcome of interest. Disadvantages of cohort studies are that they are time- and resource-consuming (289). In studies I and II, we utilized data from a prospective cohort of visitors of an STI-clinic recruited at the visit for chlamydia testing (107). The goal of initial studies was to measure chlamydia infection and associated behaviours at the inclusion and follow-up, and establish the risk factors for repeated infection at the follow-up (107). Regrettably, 44% (1 063/ 2 418) of the initial cohort did not return for a follow-up visit, which represents one of the paramount biases in prospective cohort studies, loss to follow-up (selection bias) (289). This bias and insufficient sample size prevented us to use the follow-up data. Therefore, we only utilized the data at the inclusion and analysed it as a cross-sectional cohort. We also performed a drop-out analysis, and found that married/cohabitated individuals, seldom tested for chlamydia during their lifetime, and those tested negative for chlamydia at the inclusion did drop out more often at follow-up (206).

7.3.1.2 Mathematical modelling study

In Study IV, we applied a basic deterministic model, which considered surveillance data, natural history, and health care-seeking behaviour. We obtained population estimates for averaged sexual risk behaviour across age groups and sex. With additional data on different levels of sexual risk behaviour and partnerships, it would have been possible to increase the model complexity with further parameterisation. Another limitation of the model is that it is not suitable for forecasting, thus we cannot study the impact of individual (or combined) control measures (such as PN) on incidence rates and prevalence.

7.3.2 The role of bias

It is important to consider to what degree systematic error (bias) has influenced results during the design, conduct, and analysis of the study. Several potential biases may arise in our research and are described below.

7.3.2.1 Participation bias

Participation bias (non-response bias) can arise when some factor affects which individuals participate in the study. In studies I and II, we utilized data from an STI clinic, where *volunteer bias* could arise if individuals accepting to participate in the study are in some way different from those who do not. In sexuality research, it has been reported that individuals accepting participation in a study have in general a more open attitude towards

sexuality, and are more sexually experienced compared to those who chose not to participate (290, 291).

7.3.2.2 Information (measurement) bias: Social desirability bias

In studies I and II (partly in Study IV), we used data on self-reported sexual behaviour, which is a sensitive topic. Evidence suggests that respondents in the research of sexuality and sexual behaviour are prone to report socially acceptable behaviour due to expected gender norms in society (292). This in turn can distort the association between inaccurately reported sexual behaviour and the outcome of interest. It is commonly reported that men report more sexual partners than women (293-295), not only due to the social desirability bias, but also due to the different reporting techniques applied: estimating vs. counting sexual partners (293). In our study, the median number of sexual partners during the previous 12 months did not differ by sex, although the range of sexual partners was higher for men than women. Additionally, we found that the number of casual sexual partners during the previous 12 months was slightly higher for men than for women (120).

7.3.2.3 Recall bias

In studies I and II, we used behaviour data, which respondents reported retrospectively for 6 months, 12 months and a lifetime. General recall bias can take place since study respondents may recall past events or experiences differently in terms of accuracy and completeness (296). Recall bias can also be differential if respondents recall their exposure "better" based on the outcome of the test (i.e. individuals may try harder to remember if they have a disease) (297). Overall, underreporting or inaccurate reporting of past behaviour is reported for longer recall periods as compared to the ones close in time (298, 299). This can lead to underreporting or missing data. However, in our study I and II, the questionnaire was distributed before obtaining the result on chlamydia status, therefore recall bias should not be differential.

7.3.2.4 Item response bias

This type of bias is characterized by respondents refusing to answer a particular question or set of questions. Sexual behaviour and substance use are reported as sensitive topics, and therefore item non-response is a possible consequence of it (125, 300). There is strong evidence that computer-assisted interviews can decrease item non-response to sensitive topics as compared to self-administered questionnaires or face-to-face interviews (301). In studies I and II, we used a self-administered paper questionnaire, where we aimed to minimize this bias by piloting the questionnaire on a suitable population and consequently adjusting the design of the questionnaire. Despite of this, we experienced missing values for some of the questions. This was handled by using multiple imputations (302). We also

performed item non-response analysis, where we found that the missing answers were concentrated on questions asking about the number of partners, the reason for testing, and the use of condoms with casual partners (206).

7.3.2.5 Bias due to assumptions in the model

In study III, we selected a limited number of manifest (observed) variables to identify latent classes. We believe that the sample size of our study was sufficiently large, and that we selected accurate high-quality manifest variables, i.e.variables predicting latent variables with a probability near one or zero, to identify these latent classes. However, as discussed elsewhere, it is possible to increase the number of possible patterns, which would be missed otherwise due to their rarity, by adding extra observed variables (303).

In study IV, we make a number of assumptions in the model regarding the distribution of the parameters and the data. We also had to rely on the existing incomplete knowledge on the natural history of chlamydia (304, 305), which could affect the outcome of the model. However, we performed extensive sensitivity analyses (as described in *Material and Methods*), and were able to identify the parameters that our modelling outcomes were either sensitive to or robust for.

7.3.3 Confounding

When an association between exposure (independent variable) and outcome (dependent variable) is estimated, it is important to assess whether a third variable can influence (distort) this relationship. The third variable here is a confounder if it is causally related both to the exposure and to the outcome: importantly, a confounder is not part of the causal pathway between exposure and outcome (306). Typical examples for confounders in epidemiological research are age and sex. It is possible to control for confounding at the design stage, by randomization, restriction, or matching (306). At the analysis stage, stratified analysis and multivariate analysis (regression adjustment) are possible solutions to control for confounding variables (306).

We had to control for age and sex in our studies at the analysis stage. In study I, we were applying directed acyclic graphs using a web application to visualize assumptions about confounding variables (307). This method helped us to minimize the bias in further multivariate regression, if the directed acyclic graphs we created is correct. In study II, we performed the stratified analysis by sex and adjusted for the age groups in the analysis of associations between latent classes and outcomes. In study III, the age and sex could be potential confounders when comparing the differences between the types of counties due to possible shift in age and sex composition of tested populations over time. However, available data for the period 2009-2018 suggests that age and sex distribution among the tested population did not change. In study IV,

we modelled the outcomes by stratifying by sex and age groups as some of the input parameters and the data could be confounded.

7.3.4 Generalizability

Generalizing the results from study I and II to the broader population requires care, as our study respondents were recruited at an STI clinic. It has been reported previously that a higher proportion of individuals with high-risk behaviour will be represented among the typical visitors of an STI clinic (241, 249, 308). We are potentially limited in generalizing results from our study III to countries other than Sweden. Although, it is possible that our results on chlamydia trends and associations can apply to countries with a similar healthcare system, and similar chlamydia epidemiology and control measures. Results from study IV were robust and can be generalized to other high-income countries of similar sexual behaviour in the population.

8 CONCLUSIONS

Studies I and II:

- Identified independent risk factors for incident chlamydia infection were consistent with published evidence within and outside Sweden.
- Individuals in latent classes of high-risk sexual behaviour are more likely to test repeatedly for chlamydia during their lifetime, suggesting that they complied with prevention messages aimed to increase testing after unprotected sexual contact with new or casual partners.
- Identified independent risk factors and high-risk sexual behaviour latent classes indicate the practice of unsafe sex, endorsing the need for enhanced promotion of safer sex and testing.

Studies III and IV:

- Chlamydia notification rates in Sweden followed the shape observed in other high-income countries during 1992–2004. However, Sweden stands out from other countries by having decreasing trends of chlamydia notification rates during 2009–2018.
- Chlamydia notification rates were not driven by testing during 2009–2018, as they were during 1992–2004. This suggests less biased notified chlamydia IRs, mirroring true incidence rates during the period 2009–2018.
- Estimated chlamydia prevalence was in line with prevalence in other high-income countries for both men and women.
- Estimated chlamydia prevalence declined during 2009–2018, supporting the hypothesis on being the reason for the declining observed chlamydia rates.
- The estimated high proportion of undiagnosed asymptomatic chlamydia cases is likely to contribute to the ongoing transmission of chlamydia. Thus, testing for chlamydia should expand to reach more asymptomatic individuals.

9 SUGGESTIONS FOR FUTURE RESEARCH

This thesis addressed several research questions, which were of relevance specifically to Sweden, but could be also applicable for other countries. However, several blind spots remain to be addressed.

With study I, we have contributed to the accumulated knowledge on factors associated with chlamydia. To our regret, we were unable to study sexual behaviour factors associated with repeated chlamydia infection due to the small sample size. Thus, this merits further study to inform Swedish-specific control measures and interventions.

Our study III revealed that we still do not know enough about the population tested for chlamydia. We attempted to answer this question with study II, however, visitors to an STI clinic are not entirely suitable to draw conclusions on the general population. Future research should focus on studying behavioural factors, including the reason for testing, among the general population or the population attending different types of health care services. The results would allow determining whether relevant subgroups at risk are being reached.

Opportunistic testing is the approach employed in Sweden, however, it has not been formally evaluated. Therefore, a study to evaluate the effectiveness of opportunistic testing on chlamydia prevalence reduction and prevention of sequelae from chlamydia infections is warranted, also in light of existing limited evidence internationally (157-159). With the current increasing number of tests annually, case management, and PN, the control strategy in Sweden should also be evaluated in terms of costs as was done elsewhere, for instance in the USA (309). The most recent estimation of costs in Sweden was in 2010 for the PN work alone and yielded 5.6 million EUR per year, resulting in PN costs of 150 EUR per one chlamydia case (310). Such evaluations are essential for the planning of resources.

Study IV estimated a large number of undiagnosed (including asymptomatic) chlamydia cases in the population, but there is still little understanding of effective ways to reach the asymptomatic infected individuals with testing. Thus, research should target this area to develop user-acceptable and feasible testing approaches. For instance, some research showed promising results in school or university-based testing programmes (311). Furthermore, men should be of particular focus due to their poorer test-seeking behaviour (156, 247).

Moreover, our results suggest the need for the promotion of safe sex. Future research should look into the most effective ways to target persons at risk at the population level and individual level. Population-based interventions via modern technology and social media is a popular approach that can reach a large amount of target population. However, the effectiveness and sustainability of such interventions on sexual risk-behaviour change could be limited (312, 313) and should be explored further. Based on the results from study II, more tailored prevention strategies should be explored based on the sex and risk-behaviour profile of individuals. Effective and long-lasting population-based and individual-based interventions reaching specific subgroups should be studied further as current evidence suggest that their effect could be weak and with limited length (312-314). Additionally, further research should

be encouraged to explore how individuals apply current chlamydia prevention strategies, e.g. practising both testing and condom use, or favouring one over the other.

While modelling studies can provide estimates on the outcome of interest, such as prevalence, other study types should be considered in Sweden as well. Future studies to estimate chlamydia prevalence could be carried out on a random sample of the population and using existing knowledge-attitude-behaviour questionnaires together with biological sampling for chlamydia. Such methodology successfully is applied in the UK (National Surveys of Attitudes and Sexual Lifestyles (NATSAL)) and the USA (National Health and the Nutrition Examination Survey (NHANES)) (94, 315).

Studies III and IV indicated that it is warranted to further investigate what components of chlamydia control strategy in Sweden affect chlamydia incidence and prevalence. Modelling studies could help answer those questions and develop a greater understanding of the role of different components, such as partner notification, which can inform future control strategies.

10 SAMMANFATTNING PÅ SVENSKA

Klamydiainfektion orsakas av bakterien *Chlamydia trachomatis* och är den vanligaste sexuellt överförbara infektionen (STI) i världen. Varje år tillkommer uppskattningsvis 127 miljoner nya fall världen över. På grund av infektionens asymptomatiska karaktär kan individer bära den länge och överföra infektionen utan att veta om det. Obehandlad klamydia kan leda till allvarliga följder på den reproduktiva hälsan, såsom äggledarinflammation, som vidare kan leda till utomkvedshavandeskap och infertilitet. Klamydiainfektion kan tidigt upptäckas via testning (opportunistisk screening), behandlas och smittspåras för att förhindra vidare överföring.

Syftet i denna avhandling var att få utökad förståelse för klamydiaepidemiologin på individ- och befolkningsnivå i Sverige. Avhandlingen baseras på data från en kohortstudie från en STI-mottagning, samt antal klamydiafall och antal testade personer som rapporterades till det nationella registret av smittsamma sjukdomar, SmiNet-2, på Folkhälsomyndigheten. Vi har använt oss av olika metoder för att svara på våra studiefrågor. I studierna I och II använde vi data från en kohortstudie. I studie I fann vi att de som är 20-24 år unga, haft mer än sex sexpartners under de senaste 12 månaderna, använder alkohol före sexakten, rapporterar alla typer av sexuella aktiviteter under den senaste sexuella kontakten samt testar sig på grund av smittspårning, var samtliga oberoende faktorer som löper statistiskt signifikant ökad risk för att testa positivt för klamydia. I studie II identifierade vi fyra latenta klasser (individgrupper) av beteendemönster bland män respektive tre klasser bland kvinnor. De latenta klasserna som karaktäriseras av hög risk sexuellt beteende hade statistiskt signifikant ökade tvåfaldiga odds för livstids upprepade testning för klamydia, både hos män och kvinnor. Kvinnor med hög risk sexuellt beteende hade också ökat tvåfaldiga odds för att låta testa sig upprepade gånger under föregående 12 månader. Detta indikerar att individer med högre risk för klamydia efterlevde folkhälsorekommendationer om att testa sig om det finns risk för infektion. I studie III använde vi tidsserieanalyser för att undersöka underliggande komponenter såsom tidstrender och säsongsvariationer. Vi undersökte hur de komponenterna förändrades över tid genom att jämföra två perioder: före och efter upptäckten av en ny variant av *Chlamydia trachomatis* i Sverige. Vi analyserade data nationellt och i två olika typer av län som vi grupperade efter deras förmåga att identifiera den nya varianten. Vi justerade också klamydiaincidens för testningsvolymerna. Vi fann att klamydia incidenstrenderna ökade sedan mitten av 1990-talet fram till 2004, liksom testningen ökade, vilket tyder på att ökningen av antalet fall kunde kopplas till den ökade testningen. Å andra sidan minskade klamydia incidenstrenderna under 2009-2018, trots ökade testningsvolymer, vilket tyder på att antalet upptäckta klamydiafall inte drevs av testningen, och troligen återspeglar den sanna klamydiaincidensen i befolkningen. I studie IV fortsatte vi att utforska orsaken till de minskande klamydiatrenderna (2009-2018) genom att uppskatta klamydiaprevalensen med hjälp av matematisk modellering. Vi uppskattade en minskning av klamydiaprevalensen bland 15-29 år gamla män och kvinnor under denna period, vilket stödde vår hypotes om orsaken till minskade incidens under samma tidsperiod.

Sammanfattningsvis är oberoende riskfaktorer för klamydia-diagnos i linje med tidigare publicerade studier. Dessutom tyder våra resultat på att grupper av individer med hög risk sexuellt beteende är mer benägna att testa sig upprepade gånger för klamydia, vilket tyder på att rekommendationer om testning efterföljts särskilt i denna grupp. Tillika påverkades inte klamydiatrender av testningsintensiteten under 2009-2018, vilket tyder på en sann minskning av klamydiaincidensen i befolkningen. Genom att uppskatta minskningen av klamydiaprevalensen i klamydia mest utsatta åldersgruppen 15-29 år under denna period kunde vi föreslå orsaken till rapporterade sjunkande klamydia incidens. Preventivt arbete bör fortsätta för att nå symptomfria individer med testning och informationskampanjer. Ytterligare studier bör undersöka rollen som andra komponenter i klamydiakontrollstrategin har för att urskilja deras effekter på klamydia incidens samt planera möjliga förändringar i framtida preventiva insatser.

Nyckelord: klamydiainfektion, epidemiologi, risk faktorer, incidenstalet, opportunistisk testning, upprepade testning, prevalens, matematisk modell, tidsserieanalys, latent klassanalys, regressionsanalys.

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