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**Genital *Chlamydia trachomatis* infections among adolescents in a high-incidence area in Norway: genotypes, prevalence, early sexual behaviour and testing patterns – a cross-sectional study**

**The Finnmark High School Study (FHSS)**

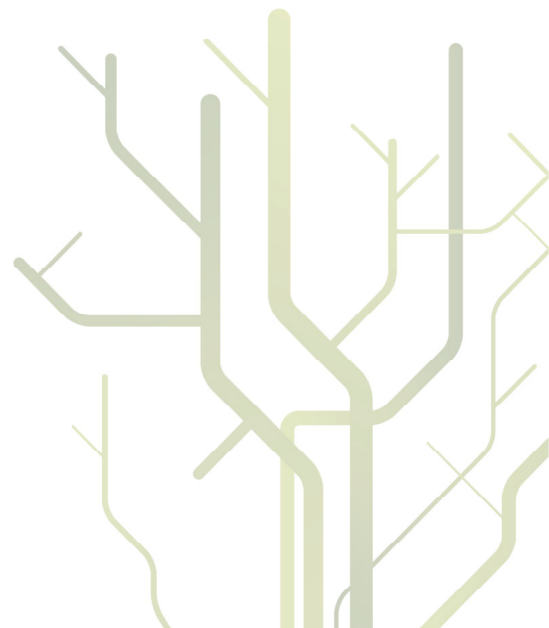


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A dissertation for the degree of

Philosophiae Doctor

2013







‘Sexual behaviour and chlamydia among high school students in Finnmark’

### **Comments heard during data collection**

‘Seriously, do you really think I’m just going to sit here and do boring school work while the others are answering that extremely interesting sex-quest – hello, I’ve changed my mind, email me that quest right away!’

‘This questionnaire is so useful to sum up my life experiences’.

‘What the f... has education and religion got to do with having a chlamydia infection?’

‘What is the problem with having something that doesn’t ever show itself?’

‘How come you ask me - a boy of only 17 - if I’ve ever been with a prostitute?’

‘Why do you only test our urine samples for chlamydia? You should check for everything!’



## **Preface**

During my years as senior physician at the Regional Centre for Infection Control at the University Hospital of North Norway, I was often approached by colleagues and by representatives from the national health authorities at meetings and conferences who inquired about the ‘chlamydia epidemic’ in Finnmark county as indicated by surveillance data. The questions would commonly be accompanied by humorous suggestions of reasons for the high chlamydia rates. Every spring, the Norwegian Institute of Public Health would publish their annual chlamydia report that listed priority tasks in the field of chlamydia prevention.

‘Increased knowledge about the chlamydia epidemiology in Finnmark’ was usually included on that list, but no relevant research studies were initiated. Eventually, I was ready to do my PhD. I realised that this was my opportunity to study genital chlamydia infections among young people in Finnmark and I started planning my PhD project. After two years of applying for funding and permissions, we finally set off to Finnmark in September 2009. We carried boxes and suitcases filled with urine sample transport tubes, disposable gloves and laboratory forms and were enthusiastically received by students and staff in 5 high schools. It turned out to be a fantastic journey. This thesis includes three papers from the Finnmark High School Study.

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## Summary

*Chlamydia trachomatis* is the most commonly reported curable sexually transmitted infection in Western high-income countries and can cause severe female reproductive tract morbidity. Despite extensive control efforts, chlamydia rates have increased in most countries since the mid-1990s. Young persons and especially adolescent girls have the highest infection rates. In general, girls are tested far more frequently than boys. High-resolution genotyping provides detailed information on the molecular epidemiology and genetic diversity of *C. trachomatis*.

In this thesis, we investigated; i) *C. trachomatis* genotype distribution and genetic diversity using MLST (multilocus sequence typing) and *ompA* genotyping in Finnmark, a high-incidence area in Norway, ii) associations between early sexual behaviour and prevalent chlamydia infection, and iii) demographic and sexual behaviour factors associated with chlamydia testing in a high school based screening and previous clinic based testing, among girls and boys aged 15-20 years who participated in the Finnmark High School Study conducted from September to November 2009.

We detected a large genetic diversity, multiple novel sequence types and alleles by MLST, and an atypical genovar distribution with predominance of G in a previously unmapped area. *C. trachomatis* genetic diversity in rural Finnmark and two other urban areas was similar. Chlamydia prevalence in sexually active girls was 7.3% and in boys 3.9%. Girls had earlier sexual debut and were more sexually active at a younger age and thus had a different risk profile from boys which may contribute to higher prevalence. Threefold more girls than boys reported previous clinic based testing which was associated with known chlamydia risk factors. School based screening reached 93% of participants and was associated with factors unknown to increase risk thus suggesting other motives. Half of infections were detected in those only tested at school. We confirmed the efficiency of school based screening to increase testing and detect hidden infections and we thus suggest this approach to be tried as a complement to other chlamydia control strategies in selected high-morbidity areas in Norway.

## Sammendrag

*Chlamydia trachomatis* er den hyppigst rapporterte seksuelt overførbare infeksjonen i vestlige land. Ubehandlet infeksjon hos kvinner kan føre til svangerskap utenfor livmoren og barnløshet. Tross omfattende kontrolltiltak har forekomsten av klamydiainfeksjoner økt i de fleste land siden midten av 90-tallet. Unge personer og særlig kvinner har høyest forekomst. Jenter tester seg generelt mer enn gutter. Genotypimetoder med høy oppløselighet kan gi kunnskap om molekylærepidemiologien og den genetisk diversiteten av *C. trachomatis*.

I denne avhandlingen har vi undersøkt; i) distribusjon og genetisk diversitet av *C. trachomatis* genotyper ved bruk av MLST (multilokus sekvenstyping) og *ompA*-typing i Finnmark som har den høyeste insidensraten av klamydia i Norge, ii) om kjønnsforskjeller i tidlig seksualatferd er relatert til prevalent klamydiainfeksjon, og iii) om demografiske faktorer og seksualatferd har betydning for deltakelse i en klamydiascreening i videregående skole og for tidligere testing i klinisk praksis blant jenter og gutter i alderen 15-20 år som deltok i en forskningsstudie ved fem skoler i Finnmark fra september til november 2009.

Vi påviste stor genetisk diversitet, mange nye alleler og sekvenstyper ved MLST, samt en atypisk genovarfordeling med dominans av G i et ikke kartlagt område. Genetisk diversitet var lik i Finnmark og to større byer. Klamydiaprevalens hos seksuelt aktive jenter var 7,3% og hos gutter 3,9%. Jenter hadde lavere seksuell debutalder og var tidligere mer seksuelt aktive enn gutter. Ulik risikoprofil kan bidra til å forklare kjønnsforskjeller i prevalens. Tre ganger flere jenter enn gutter rapporterte tidligere testing i klinisk praksis, mens testraten var 93% for begge kjønn i skolescreeningen. Tidligere testing var assosiert med kjente risikofaktorer for klamydia, mens deltakelse i screeningen var assosiert med faktorer som vanligvis ikke er knyttet til infeksjonsrisiko. Dette tyder på at andre motiver var viktige for deltakelse i skolescreeningen. Halvparten av infeksjonene ble påvist blant personer som kun testet seg på skolen. Vi bekreftet at skolescreening øker testing og påviser et større infeksjonsreservoar. Vi foreslår derfor at skolescreening utprøves i selekterte områder med høy klamydiaforekomst som et tillegg til andre forebyggende tiltak mot klamydiainfeksjoner i Norge.

## List of papers

This thesis is based on the three following papers, which will be referred to in the text by their Roman numerals:

- I. **Gravningen K**, Christerson L, Furberg AS, Simonsen GS, Ödman A, Herrmann B. Multilocus sequence typing of genital *Chlamydia trachomatis* in Norway reveals multiple new sequence types and a large genetic diversity. *Plos One* 2012; 7:e34452. doi:10.1371/journal.pone.0034452 PONE-D-11-25772 [pii].
  
- II. **Gravningen K**, Furberg AS, Simonsen GS, Wilsgaard T. Early sexual behaviour and *Chlamydia trachomatis* infection - a cross-sectional study on gender differences among adolescents in Norway. *BMC Infectious Diseases* 2012;**12**:319. doi:10.1186/1471-2334-12-319.
  
- III. **Gravningen K**, Simonsen GS, Furberg AS, Wilsgaard T. Factors associated with *Chlamydia trachomatis* testing in a high school based screening and previously in clinical practice: a cross-sectional study in Norway. *BMC Infectious Diseases* 2013, **13**:361. doi:10.1186/1471-2334-13-361

## Abbreviations

CASI	Computer-assisted self-interview
CI	Confidence interval
CDC	Centers for Disease Control and Prevention (Georgia, US)
<i>C. trachomatis</i>	<i>Chlamydia trachomatis</i>
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control (Sweden)
FHSS	Finnmark High School Study
FVU	First-void urine
IR	Incidence rate
MLST	Multilocus sequence typing
MOMP	Major outer membrane protein
NAAT	Nucleic acid amplification test
NIPH	Norwegian Institute of Public Health
nvCT	New Swedish mutated variant of <i>C. trachomatis</i>
<i>ompA</i>	Gene coding for major outer membrane protein (MOMP)
OR	Odds ratio
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
ST	Sequence type of <i>C. trachomatis</i> based on MLST
STI	Sexually transmitted infection
UNN	University Hospital of North Norway
WGS	Whole-genome sequencing

## **1. Introduction**

### **1.1 Bacteriology**

*Chlamydia trachomatis* is a small (1,000 kB) obligate intracellular bacterial pathogen with a specialised biphasic developmental cycle. The bacterium effectively conceals its antigenic profile from the immunity system by replicating in an intracellular vacuole and then moving between two hosts in the non-replicative form. It belongs to the order *Chlamydiales*, the family *Chlamydiaceae*, and the genus *Chlamydia*, which includes *C. trachomatis* that has humans as its only reservoir. *C. trachomatis* comprises two biovars: the trachoma biovar that includes ocular and urogenital strains causing localised infections of the epithelial surface of conjunctiva or genital mucosa, and the lymphogranuloma venereum biovar that can spread systemically through the lymphatic system causing genital ulcer disease. Most *C. trachomatis* strains possess a cryptic plasmid of 7.5 kB that mostly shares the same evolutionary history as their chromosomes and is putatively linked to virulence [1].

### **1.2 Clinical course**

*C. trachomatis* has a long infectious period with less than half of untreated infections resolving spontaneously within a year [2, 3]. Repeat infections in adolescents are common suggesting limited development of immunity following a first infection [4-7]. More than 95% of chlamydia infected women and men in population based studies report no symptoms [8]. In women, major clinical manifestations include urethritis and cervicitis [9, 10]. Untreated infection in women can ascend to the upper genital tract and cause salpingitis and lead to pelvic inflammatory disease with scarring and fibrosis of the affected tissues which can result in chronic pelvic pain, ectopic pregnancy and tubal infertility [11]. Other adverse pregnancy outcomes include miscarriage, stillbirth and preterm labour, although studies show conflicting results [12, 13]. Infection in men generally presents as urethritis which can lead to epididymo-

orchitis and possibly infertility [10, 14]. Current Norwegian guidelines recommend genital infections to be treated with doxycyclin 100 mg twice daily for 7 days, alternatively azithromycin one gram single dose can be used if poor compliance is anticipated [15]. As chlamydia antimicrobial assays are complex, non-standardised and difficult to interpret, antibiotic resistance is not routinely assessed in the laboratories [16, 17]. False positive test results may occur up to three weeks after treatment due to persistent DNA [18]. It may be difficult to distinguish between *C. trachomatis* treatment failure and reinfection because of the possibility of re-exposure to an infected partner [5, 19]. Non-compliance should also be considered if test of cure is positive. No vaccine against genital *C. trachomatis* infection is yet available [20].

### **1.3 Detection and typing**

Increased testing for *C. trachomatis* became possible in the 1980's when inefficient cell culture systems were replaced by direct fluorescent microscopic assays, and later by enzyme immunoassays. In the period 1996 to 1999, most Norwegian laboratories implemented the currently used nucleic acid amplification tests (NAATs) that retain both high specificity and sensitivity when applied to urine and vaginal swab specimens [21]. Culture-based techniques are no longer used in Norwegian laboratories. NAATs provide high throughput and are presently the gold standard for chlamydia detection in well resourced settings. The possibility to use first-void urine (FVU) samples in both females and males has expanded testing in non-clinical settings, including high schools.

Genotyping of *C. trachomatis* has a wide range of applications: to examine genetic population structure, as a tool in epidemiologic studies, to reveal transmission in sexual networks, to discriminate between repeat and persistent infections, to detect clonality in an outbreak investigation, and in surveillance of emerging strains such as the Swedish new variant of *C.*

*trachomatis* (nvCT) [22]. It is assumed that persons infected by the same chlamydia strain are more likely to be epidemiologically linked than those infected with different strains.

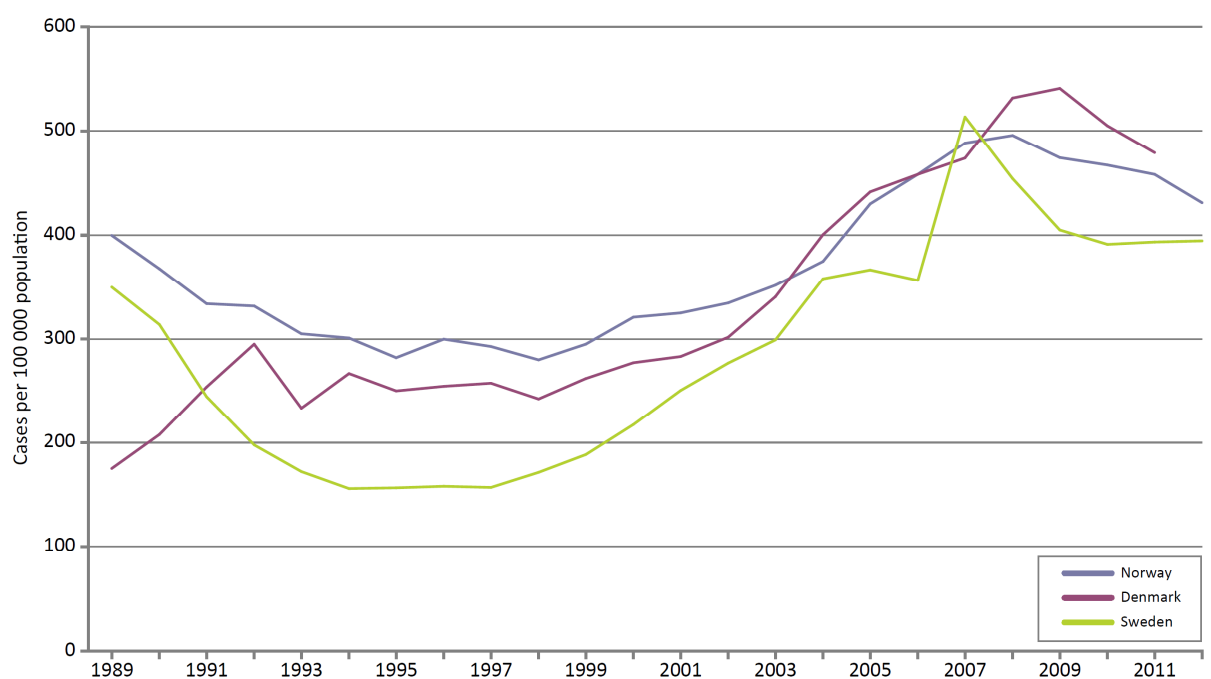
Historically, antibodies recognising the major outer membrane protein (MOMP) were used to separate *C. trachomatis* into serovars [23]. *ompA* sequencing is based on the gene encoding MOMP and has been the most widely used typing scheme in *C. trachomatis* in the past decades. It has higher resolution than immunotyping and separates chlamydia into the genovars A-C associated with trachoma, D-K with urogenital infections, and L1-L3 with lymphogranuloma venereum [24]. As the most prevalent genovar E has been detected in about half of chlamydia urogenital infections in heterosexual populations worldwide, recent research has focused on developing genotyping methods with higher discrimination [24-28].

The availability of whole-genome sequencing (WGS) has led to the development of several new genotyping systems. By 2009, four DNA typing methods for *C. trachomatis* genotyping had been published. Two different multilocus sequence typing (MLST) schemes both using 7 housekeeping genes with a resolution similar to that of *ompA* sequencing were available [29, 30]. These are most useful when exploring long term trends in evolutionary studies. In addition, two schemes with higher resolution had been described; a multilocus variable number of tandem repeats (VNTR) analysis published by Pedersen *et al.* in 2008 [31], and an MLST scheme based on 5 highly variable targets of non-housekeeping genes that was developed by Klint *et al.* in Uppsala, Sweden in 2007 [32]. This MLST scheme had been used in several studies in neighbouring country Sweden and was chosen due to high resolution and to enable comparison of sequence types (STs) sampled in the Finnmark High School Study to those included in the Uppsala University MLST database, <http://mlstdb.bmc.uu.se>. No previous studies had applied high-resolution genotyping in *C. trachomatis* samples from heterosexual persons in Norway or used it as an epidemiologic tool to examine genetic diversity in samples from a general adolescent population including both genders.



## 1.4 Epidemiology

*Chlamydia trachomatis* infection is the most commonly reported bacterial sexually transmitted infection (STI) among heterosexual persons in developed countries worldwide [33-35]. True incidence of infection is assumed to be higher than the reported numbers due to its asymptomatic nature. In Western countries, more than two-thirds of all genital chlamydia infections are detected in persons aged 15-24 years, more often in females than males [33, 34]. In 2009, a total of 344 000 cases were reported in Europe, an overall incidence rate (IR) of 185/100 000 [33]. Norway had the third highest chlamydia IR (467/100 000). As 88% of the chlamydia infections in 2009 were reported by four countries (Denmark, Norway, Sweden and the United Kingdom) the results may primarily reflect high levels of testing and thorough reporting in these countries.



**Figure 1.** Number of chlamydia cases per 100 000 population reported from the laboratories in Norway, Denmark and Sweden from 1989 to 2012 [36-39].

The chlamydia IRs in Norway have followed a similar pattern as that of Denmark and Sweden; a decreasing trend from the late 1980s to the mid-1990s, followed by a continuous

increase and more than doubling of IR until 2008, while lately a small annual reduction has been observed (Figure 1) [33, 37-39]. The peak in IR in Sweden from 2006 to 2007 was caused by the identification of the mutated nvCT with a deletion in the cryptic plasmid that included the targets for two common commercial diagnostic tests. The nvCT had thus escaped detection in the preceding years [40].

The general increase in chlamydia rates observed in many Western countries since the mid-1990s has been explained by the use of more sensitive diagnostic tests, increase in screening coverage and frequency, improved targeting of risk groups, and possibly a true increase due to changing sexual behaviour [33, 41]. The arrested immunity hypothesis introduced by Brunham *et al.* in 2005 suggests that screening may have increased reinfection rates because early detection and treatment may diminish the immune response [42].

A chlamydia IR almost twice the Norwegian average has been reported in Finnmark, the northernmost county (Figure 2), with an IR of 898/100 000 in 2009 [43].



**Figure 2.** Map of study area.

In Finnmark, the chlamydia IR has peaked in females aged 15–19 years, while in males the highest IR has been observed among the 20–24 year olds (Table 1). In contrast, the national IR has peaked in age group 20-24 years in both females and males which is in line with surveillance data from Denmark and Sweden [38, 39]. The evaluation of local prevalence data and risk factors to plan chlamydia interventions has been emphasised [44, 45].

**Table 1.** Numbers of incident chlamydia cases per 100 000 population in age group 15-19 years and 20-24 years by gender in Finnmark county and Norway in 2009 [43].

	Females		Males	
	15-19 years	20-24 years	15-19 years	20-24 years
<b>Finnmark</b>	918	584	225	419
<b>Norway</b>	313	412	81	245

### 1.5 Gender differences in chlamydia prevalence among adolescents

By linguistic definition, *sex* refers to physiological and biological characteristics, while *gender* refers to behaviours, roles, expectations and activities in society [46]. As my thesis examined gender differences in sexual behaviour and testing patterns, the word *gender* is used throughout.

The finding that girls in age group 15-19 years have higher chlamydia IRs than same-aged boys in surveillance data has commonly been explained by more screening opportunities for young women and girls more actively seeking health care [33, 34, 43]. However, a number of cross-sectional studies among adolescents in Western countries show significantly higher chlamydia prevalence in girls than in their male peers, both in school based settings in Southern Norway [47], Luxembourg [48] and the US, [49] and in the general population in

the Netherlands [50], England [51] and Germany [52]. The discrepancy has been linked to cervical ectopy with increased biological susceptibility in adolescent girls [53] and to the possibility that male-to-female transmission may be more efficient than that of female-to-male transmission [54]. In addition, social and cultural factors may contribute [49, 55]. No studies including biological specimens had assessed gender-specific associations between early sexual behaviour and chlamydia infections in age group 15-20 years prior to the Finnmark High School Study. Only a few population-based studies reported prevalence in boys this age [48-50].

## **1.6 Early sexual behaviour**

Adolescence is a period of rapid biological, mental and social development where lifestyle and behaviours with impact on future sexual health frequently are initiated. Sexual behaviour has been shown to vary over time and between cultures and to be deeply rooted in the social or gender constructs of a society [56-60]. The Nordic countries have more liberal attitudes towards female and adolescent sexuality than most other Western countries, and the sexual culture is characterised by equality between genders [61, 62]. However, Nordic data have indicated gender differences in age at first intercourse, number of coital partners, and type and amount of sexual experience [62-67]. Sexual intercourse in adolescents has been accepted provided they feel ‘mature enough for sex’, which ideally has been associated with being in love with the partner, being in a committed relationship, and acting responsible by using contraception [61, 63]. This ‘love ideology’ has traditionally been most important for girls [62, 67]. In the Nordic countries, genital intercourse has been introduced early in the stepwise accumulation of sexual experiences following culturally distinct ‘sexual scripts’ which refers to norms for when, where, and what you can do, and with whom you can have sex [61, 68, 69]. Patterns of early sexual behaviour have converged between genders, and since the early 1970s girls in the Nordic and a few other countries in Northern Europe have experienced first

sexual intercourse earlier than boys [57, 70]. Adolescent Norwegian girls have reported steady couple relationship at a younger age than boys, more frequent and regular sexual intercourse and older sexual partners [65, 66], while boys have had more varied sexual experience including casual sex and multiple partners [62, 63, 70]. In 2002, median sexual debut age among Norwegian girls was 16.7 years and in boys 18.0 years, a decrease from 17.7 and 18.5 years, respectively, since 1992 [67]. In 2011, a Nordic study reported a median age at first intercourse of 16 years among Norwegian girls indicating a further decrease [71]. A Norwegian study from 2003 found that adolescent females were as inclined as males to break the norm of being in love as the basis for a sexual relationship [67]. With a majority of girls preoccupied with older partners, adolescent boys can either enter a relationship with a younger girl not ‘feeling mature enough for sex’, or rely on multiple occasional relationships and sporadic sex, and lower coital frequency due to less access [62]. The liberated attitude towards female sexuality, combined with the average girl entering puberty at a younger age than the average boy, girls dating older partners, and having easy access to oral contraception can explain why sexual activity over the past decades has been initiated and peaked earlier in girls than boys in the Nordic countries.

### **1.7 Chlamydia surveillance**

Most European countries report some system for surveillance of genital chlamydia infections [72]. Until 2002, surveillance of chlamydia infections in Norway was based on voluntary aggregate reporting from all laboratories to the Norwegian Institute of Public Health (NIPH) [21]. In 2003, genital chlamydia infections became mandatory notifiable and part of the Norwegian Surveillance System of Communicable Diseases. Since 2005, the laboratories are required to report year of birth, gender, municipality of residence, and localisation of infection to NIPH. Our understanding of the chlamydia epidemiology in Norway is largely based on

surveillance data. As these data lack unique individual identifiers, accurate annual testing rates and repeat testing rates in the general population cannot be estimated.

## **1.8 Testing and screening**

Testing is a crucial part of any chlamydia control strategy. With mostly asymptomatic chlamydia infections, a high proportion of those infected have no physical clue to seek health care [8]. Screening is defined as testing for chlamydia to detect and treat infections in people who do not necessarily perceive themselves to be at risk or do not know if they are infected, with the intention to reduce future morbidity [72]. Two distinct screening approaches exist. Opportunistic screening implies a health professional offering a test to patients attending health care for any reason with the health professional responsible for repeating the test offer at regular intervals. Systematic screening uses registers to identify, invite and remind the target population to be tested irrespective of health service use. The screening frequency and coverage required to reduce chlamydia prevalence and its complications remains unknown [73].

In Norway, there is no official screening programme. The Norwegian guidelines recommend testing of both females and males in the presence of clinical symptoms, or if partner is infected, or in persons aged < 25 years after change of sexual partner, or in women presenting for termination of pregnancy or antenatal care [15, 74]. According to law, testing and treatment in these groups is free of charge [75]. Test of cure 5-6 weeks after treatment and notification to sexual partners over the past 6 months is recommended [15, 74].

Chlamydia testing of adolescents is widely available in Norway. The majority of testing is done in general practice and in public youth clinics which are tailored to the needs of adolescents and are present in most municipalities [76]. Youth clinics provide contraceptive counselling without parental consent and all services are free of charge. School based

chlamydia screening is not current policy in Norway. Most high schools have a school nurse available part time offering limited STI testing as part of the general health service. Specialist STI clinics are present only in large Norwegian towns. Hospital outpatient clinics in venereology and gynaecology only accept referred patients.

In Western countries having implemented chlamydia control strategies, young females are tested far more frequently than young males [33, 38, 39, 51, 77]. According to annual Norwegian surveillance data 2007-11, the average female to male chlamydia test ratio in age group 15-19 years was 4.5 to 1, and in age group 20-24 it was 2.8 to 1 [43].

More adolescent girls and particularly boys are reached if chlamydia testing is extended to high schools and other non-clinical settings [78-80]. Extensive high school based chlamydia screening and treatment programmes including both genders have been conducted in the US; in Philadelphia [49], New Orleans [81], New York [82] and San Francisco [44], with participation ranging from 52% to 65%. European high school based screenings have reported 63% participation in Southern Norway [47], 38% in Luxembourg [48], and 73% in a small vocational school study in the Netherlands [83]. A recent systematic review of chlamydia screening in educational settings found that classroom based approaches achieved the highest test rates [84]. None of the previous school based screenings had examined the behavioural factors associated with being chlamydia tested at school.

## 2. Aims of the thesis

The overall aims of the thesis were to examine multiple aspects of the high chlamydia IRs among adolescent girls and boys in Finnmark, including chlamydia genotypes, disease prevalence, early sexual behaviour, and factors associated with testing. The specific aims for each paper were:

- I. To examine the distribution of *C. trachomatis* genotypes in a general adolescent population in a rural high-incidence area in Norway, to compare chlamydia genetic diversity in this area with that of two urban regions, and to compare discriminatory capacity of two different genotyping methods; multilocus sequence typing and *ompA* sequencing.
- II. To detect chlamydia prevalence in adolescents aged 15-20 years in a high-incidence area in Norway, and to examine gender-specific early sexual behaviours associated with chlamydia infections.
- III. To determine the proportions of adolescents tested in a high school based screening and previously in clinical practice, to detect chlamydia prevalence according to testing pattern, and to examine demographic and sexual behaviour characteristics associated with testing.



### **3. Materials and methods**

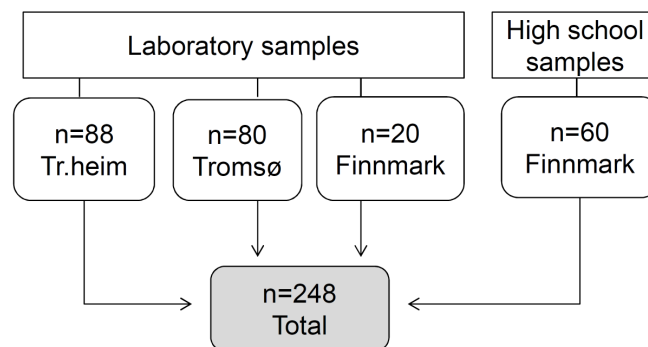
#### **3.1 Study population**

The Finnmark High School Study (FHSS) was conducted as a population based cross-sectional study in 5 public high schools in Finnmark county, Norway. Finnmark has a sparse population living in minor municipalities and borders Northwest Russia to the east, Finland to the south and east, and Troms county to the west (Figure 2). By sea, it borders the Atlantic Ocean and the Barents Sea. The population includes ethnic Norwegians, indigenous Sami people, and minority groups of Kvens, Finns and Russians. Data were collected during 9 weeks from September to November 2009 using web-questionnaires and first-void urine (FVU) samples. The principal in each school consented to participation. All 1,908 students in the high schools in the coastal municipalities Hammerfest, Kirkenes, and Alta, and in the inland Sami municipalities Karasjok and Kautokeino were invited. The student lists for each class were the basis for the invitations. All data were collected by the same experienced female doctor (principal investigator) and nurse who consecutively visited a total of 123 classes using an identical classroom based approach. In each municipality, the study staff gave tailored lectures on logistics, sexual behaviour and chlamydia infections to principals, teachers, school nurses, general practitioners and youth clinic staff prior to data collection. An invitation letter with information about chlamydia infection, questionnaire items, sampling procedures, and use of data in both Norwegian and Sami was handed out in class two weeks before data collection (Appendices 1-3). Confidentiality regarding questionnaire data and chlamydia test results was assured both in the written information and repeated orally in each class on the day of data collection. The students were informed about the mostly asymptomatic nature of genital chlamydia infections and the value of testing to prevent adverse health outcomes. The high chlamydia rates among adolescents in Finnmark were

emphasised. Chlamydia testing was promoted as a good and responsible thing to do. The testing equipment was displayed in class prior to urine sampling. Overall participation rate was 85% (1,618 of 1,908 invited students provided questionnaires and/or urine samples).

### 3.1.1 Inclusion and exclusion criteria

Paper I: The genotyping study included 60 chlamydia specimens from 1,476 urine samples with a valid chlamydia test result collected from participants in the FHSS. Parallel to the FHSS, 20 and 80 chlamydia test positive urine samples from 15-20 year olds in Finnmark and Tromsø, respectively, were consecutively collected from routine clinical samples in the laboratory of the University Hospital of North Norway (UNN Tromsø) (Figure 3). 88 samples from the same age group in Trondheim were collected at St. Olavs Hospital in Trondheim, Central Norway. Thus, a total of 248 chlamydia samples were available for genotyping.

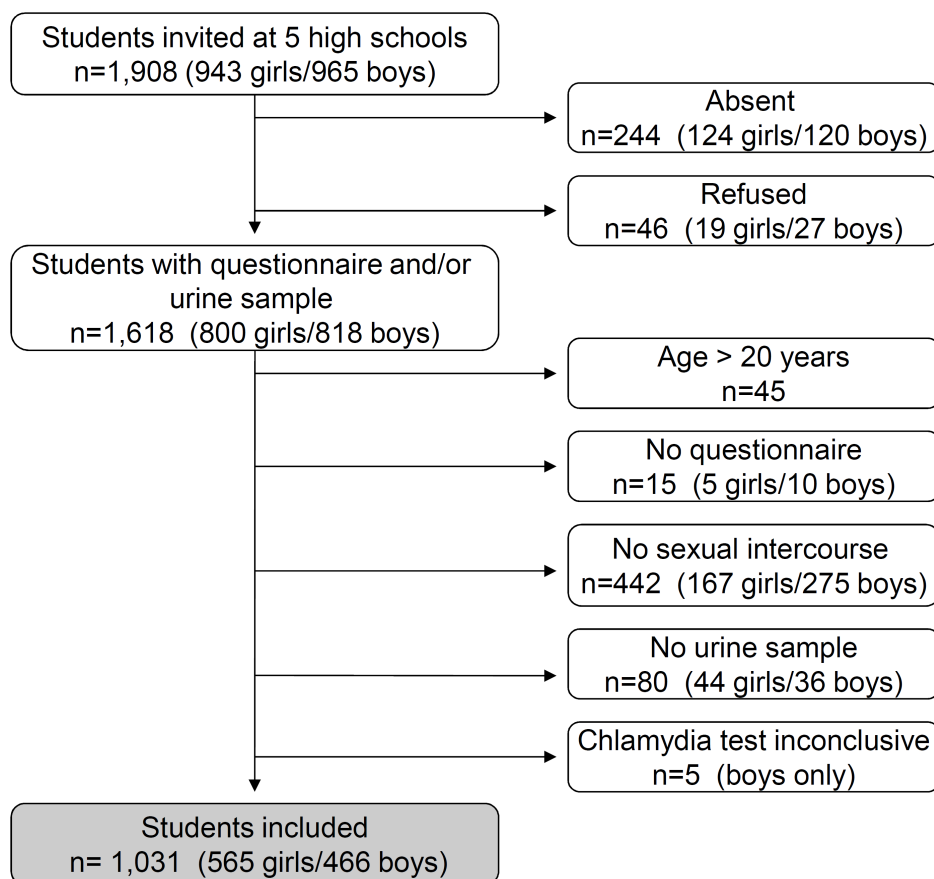


**Figure 3.** Chlamydia urine samples, Paper I.

In a separate analysis, we calculated mean age of last sexual partner in the 1,031 high school study participants with valid questionnaire and urine sample reporting sexual intercourse (Figure 4).

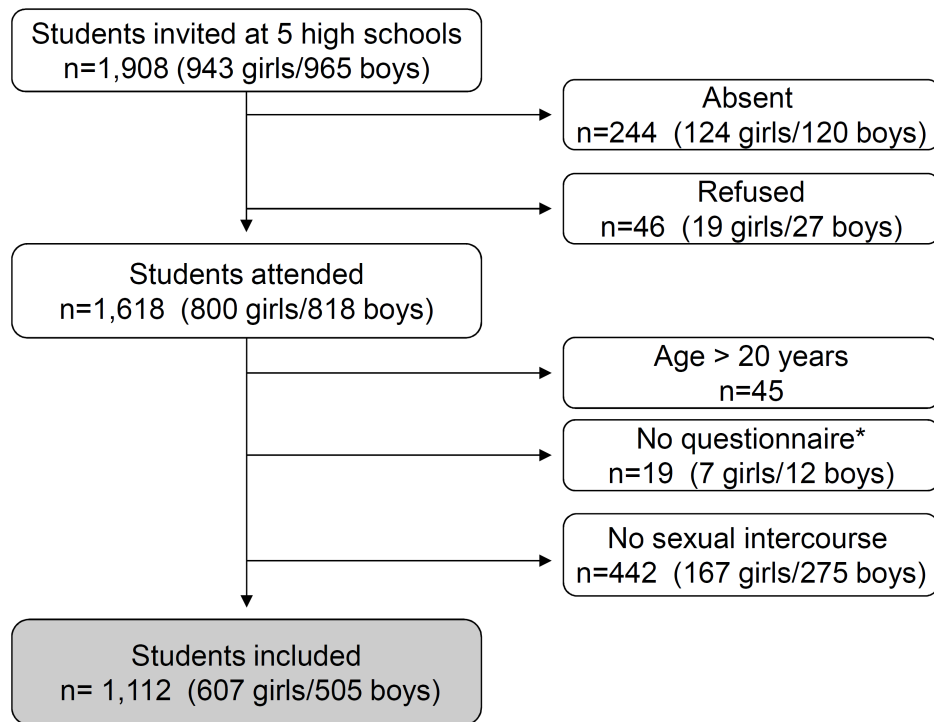
Paper II: The study population in the paper on early sexual behaviour and chlamydia infection is shown in Figure 4. If only assessing students present at school and thus eligible, 2% (46 of 1,664) refused participation. 442 participants responding ‘no’ to: ‘Have you ever had sexual

intercourse?’ were considered not to be at risk for chlamydia infection and were excluded from the analyses. All 442 had negative test results. Among 6 students with inconclusive test result, one girl testing negative one day prior to data collection was assumed to be negative and was included in the analysis. 5 boys with an inconclusive test result did not provide a new sample when asked and were excluded. A total of 1,031 participants aged 15–20 years with sexual intercourse experience, questionnaires and valid chlamydia test results were included in the study. 59 of these had a positive chlamydia urine sample.



**Figure 4.** Study population, Paper II.

Paper III: In the study of chlamydia testing, a total of 1,112 participants aged 15-20 years with questionnaires that included valid response to previous chlamydia testing, and with sexual intercourse experience were included in the analysis (Figure 5).



**Figure 5.** Study population, Paper III. \*Missing questionnaire (n=15) or missing response to the question ‘previous test’ (n=4).

### 3.2 Sample size calculations (Paper II)

We estimated a sample size of 974 to achieve 90% power to detect a difference between an anticipated chlamydia prevalence of 3.0% in the source population irrespective of sexual intercourse experience, compared to 1.4% as observed in a similar study in Southern Norway using a 5% significance level [47]. The anticipated prevalence was based on a pilot study in April 2009 in Lakselv high school in Finnmark (unpublished data).

### 3.3 Questionnaire

The questionnaire was developed for the FHSS and contained a total of 68 questions of which one third had sub items (Appendix 4 and 5). It included validated questions used in 5 nationwide surveys of sexual behaviour in 1987, 1992 and 2008 (age group 18-60 years), and 1997 and 2002 (18-49 years) [64], in a national survey of sexual behaviour in age group 17-19 years in 1989 [62], as well as in a prospective survey of adolescent sexual behaviour in Nordland County in Norway from 1999 to 2001 [85]. Ethnicity and religious affiliation was assessed using questions from the North Norwegian Youth Study 1994-95 [86]. The FHSS questionnaire was designed in QuestBack online survey system ([www.questback.com](http://www.questback.com)) and was tested for comprehensibility, clarity and time use in a pilot study including 31 students in Lakselv High School, year 1-3, in April 2009. It was adjusted according to feedback from the participants.

On the day of data collection, the questionnaire was emailed class-wise to the students 10 minutes before the study staff arrived in the classroom. All Norwegian high school students manage their own laptop computers with internet access making this approach feasible. Under supervision of the study staff and a teacher, participants spent 10-20 minutes completing the questionnaire which included questions on demography, substance use, sexual behaviour, contraceptive use, current urogenital symptoms, and earlier chlamydia testing and treatment (Appendix 4 and 5). Pre-programmed commands ensured automatic skipping of non-applicable questions. Persons with no sexual intercourse experience answered alternative questions on attitudes and feelings towards sex, intimate non-coital experiences, and STI knowledge ensuring that time spent on the questionnaire was independent of sexual experience. No reminders were sent.

### ***3.3.1 Data from questionnaires***

Self-perceived ethnicity was coded in three categories based on the statement: 'I perceive my ethnicity as: Norwegian, Sami, Russian, Kven, Finnish, or other'. Kvens are descendants of Finnish-speaking immigrants from Northern Finland and Sweden [86]. More than one answer was allowed. Category 'Norwegian' included those reporting Norwegian (n=726) and/or Kven (n=5) ethnicity, as the two share a common distribution of lifestyle factors [87]. 'Sami/Sami-Norwegian' included those reporting Sami ethnicity (n=90) or Sami and Norwegian ethnicity (n=139). 'Other' included Russian (n=19), Finn (n=20) and other (n=31) ethnicity.

Participants' residence during school year was reported as: 1) At home with my parents, 2) Living with grandparents/other relatives, 3) Private room/apartment, 4) Student house, 5) Host family, or 6) Other. Due to small groups, the variable 'Residence during school year' was dichotomised as: 'At home with my parents' (response 1) and 'Other' (responses 2-6).

The variable 'high school study affiliation' was defined as; 1) 'academic', including students in the general academic studies programme, and 2) 'vocational', including vocational school students. In Norway, academic and vocational classes frequently share facilities throughout high school.

Use of alcohol, cannabis, amphetamine or ecstasy was reported for each substance as: never tried (1), tried (2), occasional use (3), or regular use (4). A new variable 'alcohol/drug use' was calculated as sum of the four substance use variables. Participants with missing response for alcohol (n=5) were excluded, but this was accepted for the other three. Range of the 'alcohol/drug use' variable was 2–16, and was defined as:  $\leq 5$ : 'low'; 6: 'medium';  $\geq 7$ : 'high'.

Young age at first intercourse was defined as  $\leq 14$  years in accordance with a recent study assessing risk-taking behaviours among Nordic women [88].

Condom use at first intercourse with first partner and at last intercourse with last partner were coded in two categories (yes/no) based on the question: ‘Did you use any contraception at first (last) sexual intercourse?’ with response alternatives: 1) No, 2) Condom, 3) Hormonal contraception, 4) IUD, 5) Both condom and other contraception, 6) Emergency pill, 7) Coitus interruptus, 8) Don’t know. Category ‘yes’ included participants with response 2) or 5). ‘No’ included the remaining responses. ‘Don’t know’ was answered by 3 girls and 10 boys at first intercourse, and by 3 girls and 8 boys at last intercourse.

Previous clinic based testing was assessed by: ‘Have you previously been tested for genital chlamydia infection?’ with response options: ‘Yes, once’, ‘Yes, twice’, ‘Yes, 3 times’, ‘Yes  $\geq 4$  times’, or ‘No’. Due to small groups, the variable ‘clinic based testing’ was dichotomised as yes/no. We assumed all previous testing to be clinic based, i.e. youth clinics and general practice and only occasionally in STI clinics and hospital outpatient clinics. ‘School based screening’ included all participants that were screened in the high school study independent of clinic based testing. The subgroup ‘school-only test’ included participants with no previous clinic based testing that provided a urine sample in the school based screening.

### **3.4 Collection of urine samples**

After finishing the questionnaire in the classroom, participants went directly on to the school toilets where they were instructed how to provide a first-void urine (FVU) sample by the study nurse. Each participant received a test kit that included: 1) a completed laboratory form including three adhesive labels with name, birth date, and mobile phone number, 2) a urine collection cup with an ink mark at 12 ml, 3) a urine sample transport tube, and 4) disposable gloves. The nurse collected the urine transport tubes immediately outside the toilet and ensured that each person approved the printed personal information on the form and on the transport tube label. The urine samples were refrigerated and transported by National Mail

Delivery on the same afternoon to UNN Tromsø and analysed within 24 hours. UNN Tromsø is the only laboratory for microbiology diagnostic services in Finnmark.

### **3.5 Follow up**

Participants testing positive or inconclusive for chlamydia were phoned by the study nurse on the same afternoon as the laboratory reported the test result. After repeat calls, all were eventually reached and given an appointment at the local youth clinic. All the 60 test positive participants either got a prescription of a single dose one gram azithromycin or were given antibiotics directly for observed treatment. The youth clinic notified, tested and treated sexual partners. All study participants were included in a lottery with three persons winning a mobile phone with a one year subscription worth 140 Euros in 2009.

### **3.6 Laboratory testing**

#### ***3.6.1 Chlamydia PCR***

The UNN laboratory extracted DNA using the BUGS'n BEADS TM-STI kit (NorDiag ASA, Oslo, Norway) and used ProCt real-time PCR (ProCelo A/S, Tromsø, Norway) with sensitivity 97% and specificity 100% (*incA* gene and internal control). The St. Olavs Hospital's laboratory prepared DNA using the bacterial protocol on GenoM (Qiagen, Hilden, Germany) and used an in-house triplex real-time PCR (cryptic plasmid, MOMP gene and internal control) with sensitivity 96% and specificity 100% [89]. A plasmid specific PCR was used to confirm MLST identification of nvCT [40].

#### ***3.6.2 Chlamydia trachomatis genotyping***

All the 248 chlamydia samples were immediately frozen at -70°C in the laboratories and later transported on dry ice to the University Hospital of Uppsala, Sweden, for genotyping. *ompA* sequence determination was performed according to a previously described method [90].

Strains were categorised into genovars D-K and *ompA* genotypes. Genovars are *C*.



*trachomatis* subgroups based on serospecificity for MOMP inferred from *ompA* sequencing. Genotypes are subgroups based on *ompA* sequencing. The MLST scheme comprising 5 highly variable target regions was performed according to Klint *et al.* [32] and modified according to Jurstrand *et al.* [91]. Each sequence type (ST) is based on 5 digit strings that represent the different alleles. Allele profile numbers were assigned by comparing the sequence at each locus to the Uppsala University *C. trachomatis* database (<http://mlstdb.bmc.uu.se>). New allele numbers were assigned in order of discovery. Clonal complexes were defined as clusters of STs with only one allele difference, i.e. single locus variants (SLVs). The founder of a clonal complex was the ST with the highest number of SLVs.

### 3.7 Statistical methods

#### *Paper I*

The discriminatory power ( $D$ ) of a typing method, the probability that two unrelated strains sampled randomly from a test population will be categorised in different groups, was calculated for *ompA* genotyping and MLST in the 188 routine clinical samples. We used Hunter and Gaston's modification of Simpson's discriminatory index [92]:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s n_j(n_j - 1),$$
 where  $N$  is the total number of strains tested,  $s$  is the total

number of different types, and  $n_j$  is the number of strains belonging to the  $j$ th type.

Confidence interval (CI) for  $D$  was calculated as originally described by Simpson [93]. The guidelines outlined by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) state that a molecular typing method should have a  $D$  of  $\geq 0.95$  to be considered 'ideal' [94]. As the 188 samples were consecutively collected in the laboratories from a defined age group within a limited time frame from defined geographic areas, some epidemiological dependence could not be excluded. In order to adjust for this possible dependence, the following corrections were made: the two most common STs were assumed

to have prevalence equal to the third most prevalent ST. Thus, the number of strains in ST12 (n=35) and ST56 (n=28) were set equal to the number of strains in ST153 (n=15), and a corrected  $D_c$  was calculated.

Minimum spanning trees were generated by an analysis of the full MLST profiles in all 248 specimens using BioNumerics software (version 6.01, Applied Maths, Sint-Martens-Latem, Belgium) under the categorical coefficient of similarity and the priority rule of the highest number of SLVs.

Chi-square test was used to assess associations between urogenital symptoms and STs and clonal complexes. The 95% CI for proportions was calculated using Clopper-Pearson's exact method.

### ***Paper II and III***

Descriptive characteristics were reported with means (standard deviation) for continuous variables and with numbers (%) for categorical variables. The 95% CI for proportions were calculated using the exact binominal method. Crude and multivariable logistic regression models were applied using chlamydia test result (positive/negative) as the outcome variable in Paper II. In Paper III, two outcome variables were used: 1) clinic based testing, i.e. if participants had been chlamydia tested before the FHSS (yes/no), 2) school based screening, i.e. if participants were tested in the FHSS (yes/no). Variables with  $p$  value  $<0.25$  in crude analysis were included in the multivariable regression models which were fitted using stepwise procedures. Age and gender (if applicable) were included regardless of significance. Collinearity was not a problem with variance inflation factor (VIF)  $<2.5$  for all variables. Gender interaction was assessed by including cross-product terms between each independent variable and gender. All statistical tests were two-sided using a 5% significance level. SPSS version 18.0 (Paper I) and SPSS 19.0 (Paper II and III) were used for all statistical analyses.

In Paper III, one statistically significant interaction term was included in the final multivariable model, and model fit was assessed using Hosmer and Lemeshow goodness-of-fit test with 5 of 6  $p$  values  $>0.25$ .

### **3.8 Ethics**

In the FHSS, written informed consent was obtained from the next of kin, carers or guardians on the behalf of participants younger than 16 years. Participants  $\geq 16$  years gave their informed consent by filling in the web-based questionnaire in accordance with the Health Research Act §17.b stating their right to consent. All procedures were approved by the Regional Committee for Medical and Health Research Ethics North Norway (REC North No.: 200900528-6/MRO/400) and the Data Protection Officer at UNN (Number 2009/2475). Establishment of a research bio-bank for *C. trachomatis* urine samples was approved by The Norwegian Directorate of Health (Bio-bank Registry Number 2723).

## 4. Summary of results

### ***Paper I: Multilocus sequence typing of genital *Chlamydia trachomatis* in Norway reveals multiple new sequence types and a large genetic diversity***

In 248 specimens from the previously unmapped areas Finnmark, Tromsø and Trondheim, *ompA* sequencing detected 11 genotypes while MLST displayed 50 sequence types (STs) thus providing 4.5 higher resolution. A total of 12 alleles in the MLST scheme and two-thirds of all STs were novel. The common genovar E comprised 46% of all specimens and resolved into 24 different STs. MLST identified the new Swedish variant of *C. trachomatis* not discriminated by *ompA* sequencing in 1.6% of samples. Simpson's discriminatory index, *D*, for MLST was 0.93 (95% CI 0.91-0.95), while the corrected index, *D<sub>c</sub>*, was 0.97 (0.96-0.98). For *ompA* sequencing, *D* was 0.67 (0.61-0.73). There were no statistically significant differences in genetic diversity of STs between the three areas. Finnmark had an atypical genovar distribution with G being predominant, mainly due to the expansion of ST128 and the novel ST161. The latter was unique for Finnmark.

### ***Paper II: Early sexual behaviour and prevalent *Chlamydia trachomatis* infection***

Prevalence of chlamydia infection was 5.7% (95% CI 4.4-7.3%). Girls were twice as likely to be infected as boys, 7.3% (5.3-9.7) versus 3.9% (2.3-6.0). Girls reported significantly earlier sexual debut, older sexual partners, more steady relationships, higher lifetime number of sexual partners, and less condom use at last sexual intercourse than boys. Boys reported higher levels of substance use overall and in connection with last intercourse. In girls, higher maternal education (odds ratio, OR, 2.22, 95% CI 1.13-4.37),  $\geq 2$  sexual partners past 6 months (OR 3.59, 1.76-7.32), and partner meeting venue at a private party, bar or disco (OR 4.99, 1.10-22.69) increased the odds of infection in the multivariable model. In boys, condom use at first intercourse (OR 0.06, 0.01-0.42) decreased the odds of infection, while having an older last sexual partner (OR 3.74, 1.27-11.01) increased the odds. In girls and boys

combined, the risk of infection increased if residing outside the family home during the school year (OR 2.04, 1.17-3.57),  $\geq 2$  partners past 6 months (OR 2.88, 1.60-5.18), and meeting last sexual partner at a party, bar or disco (OR 3.54, 1.18-10.61), and decreased if condom was used at last intercourse (OR 0.23, 0.07–0.75). In Table 3, the correct values for ‘meeting last partner on the Internet’ for girls and boys combined should be OR 2.81 (0.78-10.08).

***Paper III: Factors associated with Chlamydia trachomatis testing in a high school based screening and previously in clinical practice***

56% of girls and 21% of boys reported previous clinic based testing. In the school based screening, 93% were tested with no gender difference. 42% of girls and 74% of boys were tested for the first time at school (‘school-only test’). Both girls with clinic based testing and girls with school-only test had high chlamydia prevalence (7.3% vs 7.2%). Boys with clinic based testing had twice the prevalence of boys with school-only test (6.2% vs 3.0%,  $p=0.01$ ). Half of infections were detected in participants with school-only test. One-fifth were repeat infections. In multivariable analysis of girls and boys combined, the following variables increased the odds of clinic based testing: older age (OR per year 1.54, 95% CI 1.30-1.83), first intercourse  $\leq 14$  years (OR 2.02, 1.43-2.85), no condom use at first intercourse (OR 1.48, 1.09-2.01), steady relationship (OR 1.51, 1.11-2.01), and higher number of lifetime partners: 1-2 partners (reference), 3-5 (OR 3.07, 2.11- 4.46), and  $\geq 6$  (OR 7.63, 5.03-11.55). Significant interaction was present between gender and ethnicity ( $p=0.012$ ). In all ethnic groups, females had higher odds of previous test than males (females versus males): Norwegian (OR 7.96, 5.26-12.04), Sami/Sami-Norwegian (3.62, 1.92-6.82) and other (OR 1.89, 0.66-5.45). In the multivariable analysis with school based screening as outcome variable, the following variables decreased the odds: female gender (OR 0.57, 0.34-0.97), vocational affiliation (OR 0.51, 0.30-0.87), first intercourse  $\leq 14$  years (OR 0.58, 0.35-0.95), and no condom use at first intercourse (OR 0.57, 0.35-0.94). In addition, current urogenital symptoms (OR 3.23, 1.57-6.65) increased the odds of school based screening.

## **5. Discussion**

The FHSS was the first high school based chlamydia screening in Europe to include both girls and boys in all three year levels and to use a comprehensive questionnaire to assess sexual behaviour. Our study was unique in applying an interdisciplinary approach that included public health aspects, mapping of sexual behaviours and testing patterns, detection of chlamydia in high quality biological samples, and the use of an advanced high-resolution method to genotype *C. trachomatis*. The FHSS was limited by cross-sectional design and self-reported questionnaire data. The study had low statistical power to assess associations between demographic and sexual behaviour factors and chlamydia infection due to the small number of chlamydia positive urine samples.

### **5.1 Internal validity**

An internally valid effect is one that correctly describes the association between exposure and outcome in the target population. Three types of systematic errors may threaten the internal validity: (i) selection bias; distortions resulting from procedures used to select subjects and from factors that influence study participation, (ii) information bias; different consequence of errors in measurement of exposure and/or disease in subjects, and (iii) confounding factors; the extraneous factors responsible for difference in disease frequency between exposed and unexposed.

#### **5.1.1 Selection bias**

Selection bias occurs if there are systematic differences in the exposure status between participants and non-participants in the study. High participation may reduce the potential for selection bias. In Finnmark county, 94% of the birth cohort was enrolled in high school from 2007-09 with an annual drop-out rate of approximately 10% [95]. An estimated number of 167 persons were lost due to drop-out throughout high school and were thus not included

(calculations not shown). Studies differ as to whether drop-outs are at increased STI risk [96, 97]. We may have underestimated levels of risk behaviours and chlamydia prevalence if drop-outs and other non-attendees had higher prevalence than the high school students.

Among non-attendees, 244 students were absent from school when the study was conducted due to excursions, field work, job training, disease or other reasons and were thus not eligible (Figure 4). Only 2% (46 of 1,664) of eligible students refused participation for unknown reasons, thus limiting the potential for selection bias [98].

### **5.1.2 Information bias**

Information bias refers to bias related to instruments and techniques used to collect information about exposure and outcome variables [99]. Differential misclassification may occur if the misclassification of exposure is associated with outcome status. The high school study participants did not know their chlamydia test result when filling in the questionnaire. Differential misclassification is possible, but we had no reason to believe there was a high level of this bias. Non-differential misclassification may occur when all categories of a variable (exposure, outcome or covariate) have the same probability of being misclassified for all participants.

The urine sampling and labelling procedures in the FHSS ensured correct linking between the persons' identity and the urine sample for each participant. Exchange of urine samples between participants is unlikely due to thorough supervision.

False positive chlamydia test results in the 60 high school samples (Paper I, II, III) and in the 188 clinical routine samples (Paper I) are unlikely as all 248 *C. trachomatis* specimens were successfully genotyped using *ompA* sequencing and MLST, providing evidence for presence of chlamydia DNA in these samples. DNA contamination in the genotyping laboratory in Uppsala is unlikely due to the finding of multiple different STs (52% of STs comprised only

one specimen and 62% had <4 specimens). Batches of specimens from all three geographic areas were analysed simultaneously, and the finding of the unique ST161 in multiple samples from Finnmark also suggests no contamination.

False negative chlamydia test results in girls are possible because self-sampled vaginal swabs in females have about 10% increased sensitivity compared to FVU samples [100]. The true chlamydia prevalence in girls may thus be higher than the estimated 7.3% (41 cases among 565 girls) with approximately four chlamydia infections remaining undetected giving a prevalence of 8.0% (45 of 565). Furthermore, the reported 97% sensitivity for the PCR test at UNN Tromsø may indicate that we have missed approximately two chlamydia cases in the FHSS. False negative test results in both genders could be caused by sampling error, transport conditions, low bacterial load, laboratory error, and PCR inhibitors. To avoid false negative test results, both the laboratories in Tromsø and Trondheim used an internal amplification control, and positive and negative controls were used both for extraction and setting up the PCR.

Obtaining a complete MLST profile in all 248 specimens was unusual because it implied every single allele PCR returning a valid result (Paper I). The result is plausible because we only used high-quality specimens that were frozen immediately after the initial diagnostic PCR. Additionally, the MLST scheme had been optimised since 2007 and analyses were performed and supervised by an experienced laboratory scientist who was very familiar with the method.

Accuracy of retrospective self-reported data depends on the participants' ability to recall past behaviours and their willingness to report them [99, 101]. Recall bias refers to differences in the accuracy of the recollections retrieved by participants [99]. All our data were self-reported, except for school year, study affiliation, chlamydia prevalence and genotyping



results. To minimise recall bias, the questionnaire proceeded chronologically from sexual debut to the most recent sexual intercourse enabling the respondents to sequentially order recall of events and promote thoughtful response (Appendix 4 and 5) [101, 102]. Personal experiences with high emotional impact such as the question on first sexual intercourse can produce ‘flashbulb’ memories and may be reported with high accuracy [102]. Single-event recall like last sexual intercourse has shown to be valid representation of sexual behaviours over longer time periods and was used in the questionnaire [103]. A number of sexual behaviour questions were linked to the first and most recent sexual partner because the ‘by-partner’ approach provides a context and a focus for past events with the potential to reduce recall bias [104].

The use of laboratory data to assess the outcome variable ‘clinic based testing’ instead of a questionnaire would have eliminated recall bias for this variable. However, recalling autobiographic events is easier if memory contains few similar events such as chlamydia testing [102]. Some recall bias in retrospective reports of sensitive behaviours is to be expected [104]. If recall bias were similar among the infected and non-infected participants, the resulting information bias will be non-differential.

Social desirability bias refers to over-reporting of socially desirable behaviours and under-reporting of undesirable behaviours thus aiming for positive evaluation by others, protecting ones’ self-image, and conforming to cultural norms [98, 101]. Our questionnaire included multiple potentially sensitive topics. We therefore used several techniques to reduce social desirability bias that included; i) priming participants’ motivation to be honest, ii) computer based self-administration of the questionnaire, iii) confidentiality assurances, and iv) careful wording of the questions [98, 105].

- i) The oral information emphasised that high quality in research depends on full honesty in reporting. We further stressed that accuracy in reporting would provide policy makers with valuable data to develop STI programmes that might benefit their age group.
- ii) Adolescents may have higher acceptability for answering sensitive questions in a computer-assisted self-interview (CASI) than in a face-to-face interview [104, 106-108]. CASI may also increase accuracy in reporting and motivation to complete the survey. The question assessing number of sexual partners seems to be sensitive in different directions between genders with women under-reporting and men exaggerating, but use of CASI has been shown to reduce such gender disparities in reporting [108, 109]. In Paper II, girls and boys reported similar numbers of sexual partners past 6 months. Significantly more boys than girls reported 1-2 lifetime number of partners (48% vs 35%,  $p < 0.001$ ), while more girls than boys reported  $\geq 6$  lifetime partners (34% vs 25%,  $p = 0.003$ ). It is likely that some gender-related social desirability bias was present in the sexual behaviour questions. However, we assume it to be smaller than in studies from Southern Europe and the US due to more liberated attitudes towards adolescent and female sexuality in Norway and to the use of CASI. Furthermore, we observed an expected association between number of sexual partners and chlamydia infection in both girls and boys suggesting high validity of the data.
- iii) Confidentiality towards parents, teachers and researchers regarding data handling, storage and analysis was assured both in the oral and written information (Appendices 1-3). We stressed that time spent on the CASI was independent of sexual experience due to design and skipping patterns. The following measures were implemented to increase levels of perceived confidentiality in the classroom during the survey: space between students, use of a CASI with small font readable only at close range, and the presence of three adults. High and universal participation with few missing responses indicates high level of perceived

confidentiality [98]. Accuracy in reporting was indicated by few extreme values in numerical variables and high level of consistency between variables.

iv) Accurate reporting of sensitive behaviours may increase by having a long introduction to the question and thus deliberately loading it [101]. The question assessing reasons for having first intercourse was phrased as follows: ‘There are usually many different reasons for a person to have sexual intercourse. What was your reason to have your first intercourse?’ The words ‘usually’ and ‘many different reasons’ may decrease the significance of the behaviour and increase the respondent’s willingness to report on it.

Item response rate was high throughout the questionnaire. Sexual behaviour topics did not suffer from low response rates with 99% answering the question on same-sex experiences and 97% replying to the question on first sexual intercourse. The detection of chlamydia infection only in participants reporting sexual intercourse suggests truthfulness in reporting. This contrasts studies in the US where detection of STIs in adolescents claiming no sexual intercourse experience is common [82, 110].

### ***5.1.3 Confounding***

A confounder is defined as a factor that blurs the observed effect and is associated both with the exposure and the outcome [99]. In contrast to selection and information bias, measured confounders can be controlled for in the statistical analysis. All multivariable analyses in Paper II and III were adjusted for gender and age. Gender interaction was assessed for all variables in crude and multivariable analyses, and significant interaction terms were included in the models. Confounding by unknown factors such as the number and timing of concurrent partnerships could not be ruled out [111-113].

## 5.2 External validity

External validity pertains to the ability to generalise the findings in the study to the general population [99]. To our knowledge, no population based study is currently available for comparison of the ST distribution in the 60 high school urine samples as the MLST scheme so far only has been used in chlamydia samples from patients attending clinical settings (Paper I) [114, 115]. When assessing all 248 samples, the findings that the putative founders of clonal complexes already were present in the MLST database and that a majority of samples belonged to clonal complexes, correspond to MLST databases for other bacteria and thus suggest high external validity [116]. Furthermore, the low prevalence of nvCT corresponds to the limited spread observed in Southern Norway and in other countries also indicating high external validity [117, 118]. Genovar E comprising 46% of all 248 samples resembles other studies on genovar distribution in heterosexual populations worldwide, and thus supports the generalisability of our results [24-28, 114].

The CASI included validated questions that had been used in comparable populations [62, 64, 67, 85, 86]. As the FHSS was confined to a chlamydia high incidence area, we assume levels of sexual risk behaviour and chlamydia prevalence to be higher than in the general adolescent population in Norway (Paper II) [47]. However, the observed gender differences in adolescent sexual behaviour are similar to reported results in other Nordic studies [62, 63]. The majority of risk factors associated with prevalent chlamydia infection (Paper II) correspond to those observed in other high school studies reporting on sexual behaviours [47, 48, 82]. To our knowledge, the association between higher maternal education and chlamydia infection in girls has not been observed in other studies. We found no previous studies assessing the association between residence outside the family home during high school and prevalent infection. Education and residence will be further discussed in the next section. The gender difference in chlamydia prevalence resembles that observed in population based studies

among adolescents in Northern Europe [47, 48, 50]. The external validity of FHSS would have increased if high school students from other parts of Norway had been included.

### **5.3 Discussion of main results**

Systematic errors such as selection bias, information bias or confounding are not likely to explain our main findings in the FHSS. In statistical models, real associations can be missed because of low statistical power, and reported associations may be spurious due to multiple statistical tests. It is likely that we did not detect some real associations due to limited statistical power. In Paper II with 59 chlamydia cases in 1,031 participants, we had 80% power to detect a population OR of 1.94 when comparing two groups of equal size.

In the FHSS, information on demographic characteristics, exposures and outcome variables was obtained simultaneously from all individuals within a narrow time period of 9 weeks. Some exposures such as age at first intercourse, condom use at sexual debut, and lifetime number of sexual partners reflect earlier exposures. In a cross-sectional study, only associations between variables can be assessed, and temporality or causality cannot be inferred [99]. Previous chlamydia test results could have influenced later sexual behaviour in the direction of less or increased risk causing a slight attenuation in the observed odds ratio estimates.

Infectious disease epidemiology has some unique features: a case may also be a risk factor, and a case may be a source without being recognised as a case due to asymptomatic infection [119]. Unprotected sexual intercourse with an infected subject is required for the occurrence of effect; a positive test result for *C. trachomatis*. As an infected subject is a source of disease in others, contact patterns in society, i.e. who meets whom, how do they meet, and how often, are important issues in order to understand the chlamydia epidemiology in a population [119].

### 5.3.1 *C. trachomatis* genotyping

Paper I describes the application of a high-resolution typing method in 248 *C. trachomatis* samples; 60 samples from the adolescent general population in Finnmark and 188 samples from the clinical laboratory routine in Trondheim, Tromsø and Finnmark (Figure 4). We detected a striking genetic diversity; 50 different STs with more than half observed in single individuals only, while two-thirds were found in less than four individuals. Multiple STs and alleles were novel. This is in line with a recent study using this MLST scheme on chlamydia samples from 52 heterosexual STI clinic patients in an previously unmapped area [114], and has also commonly been observed in databases for other bacteria [116]. The minimum spanning tree analysis showed that most STs belonged to clonal complexes which corresponds to other bacterial MLST databases [116]. MLST displayed a 4.5 higher resolution than *ompA* sequencing revealing a larger genetic diversity.

The use of Simpson's diversity index,  $D$ , provides a method to calculate the probability that two strains sampled at random from the test population will be placed into different typing groups and has most value for large and non-local collections of strains [93]. As the 60 high school samples were collected from adolescents in 5 schools during 9 weeks and the high prevalence of specific STs indicated presence of shared sexual networks, it was likely that some transmission of *C. trachomatis* infections between study participants had occurred, thus violating the criteria of epidemiological independency. We therefore calculated Simpson's  $D$  only in the 188 routine clinical samples. There may also have been a dependency structure in these samples due to the sampling context in the laboratories (short time period, only age group 15-20 years, defined geographic areas) which could lead to an underestimation of the diversity index  $D$ . Accordingly, both  $D$  and an adjusted  $D_c$  were calculated.

Only 1.6% of specimens were identified as nvCT that caused a clonal outbreak in Sweden in 2007 [22, 40]. The low prevalence was as expected because nvCT has rarely been found

outside Sweden [22, 117, 118]. After appropriate laboratory testing was introduced in Sweden, a selective decline of 24-40% of nvCT occurred in several counties suggesting a further decreased potential for nvCT to spread across international borders [120, 121].

Genovar E comprises about half of genital chlamydia infections in heterosexual women and men, a distribution which has appeared stable over time and geography [24-28]. Genovar G has been associated with infection transmission in networks of men who have sex with men [115, 122]. The FHSS was the first to report an atypical genovar distribution with G comprising 36% in a mainly heterosexual adolescent population. This was mostly due to the high occurrence of ST128 and the unique ST161. We assumed that ST128 and ST161 had expanded in local sexual networks in Finnmark, but lack of sexual network data hampered further investigation. ST161 has not been identified in chlamydia samples from other catchment areas analysed in Uppsala, Sweden, after our study in 2009 (Björn Herrmann, personal communication).

The *ompA* gene is shown to undergo extensive recombination [1, 24, 123], but the frequency of recombination events is yet unknown (Nicholas R. Thomson, the Sanger Institute, UK, personal communication). Recent whole-genome sequencing (WGS) studies by Harris *et al.* found that *ompA* has been transferred between phylogenetically unrelated *C. trachomatis* strains on several occasions providing evidence of its unsuitability as a marker for strain typing [1, 19, 124].

We found ST12, ST128 and ST161 to be the most frequent and thus most successful genotypes in Finnmark. Their success could be explained by a high transmission rate or by these STs causing a silent infection. However, only half of these infections were asymptomatic. As only 60 samples carried behavioural data, the reasons for the success could not be further elucidated. ST distribution in sexually active adolescent populations needs to be

studied in larger populations with more chlamydia samples and inclusion of partner characteristics and sexual network data.

We concluded that the MLST scheme is a valuable tool for studying the molecular epidemiology of *C. trachomatis* infections and far superior to *ompA* sequencing in terms of resolution. The MLST method has later been modified by the use of shorter target regions and nested PCR for increased sensitivity [114]. Stability and reproducibility of the 5 targets after multiple passages of nvCT in cell culture have also been documented [125]. The method is still too labour intensive to be used for partner notification in clinical practice, but next generations' sequencing technology may increase speed and make it more affordable.

All MLST schemes are limited by the fact that they only use a small fraction of the genome, and samples that are indistinguishable with respect to the 5 target regions may have considerable variation in the remaining DNA [94]. MLST only occasionally detects mixed chlamydia infections. The ultimate chlamydia typing method would be WGS with robust and finished sequences that are generated economically and quickly. A recent study showed that it is now possible to obtain WGS directly from clinical samples without culture [123]. As WGS is becoming cheaper, faster, high throughput and more available, it may in the future be one of the preferred genotyping methods and be performed in samples from clinical practice [19]. However, getting enough DNA for WGS will always be challenging, and future genotyping using WGS may be developed in two phases. First, gathering a large number of samples with as high diversity as possible to obtain WGS data. Second, from WGS data develop an SNP (single nucleotide polymorphism) based typing scheme with suitable resolution for molecular epidemiology. The SNP-scheme will be preferable to WGS if there is limited DNA (N. R. Thomson, personal communication).



### ***5.3.2 Early sexual behaviour and chlamydia infection***

In Paper II, we detected significant differences in sexual behaviour between genders. Chlamydia prevalence among sexually active girls was 7.3% and among boys 3.9% in agreement with the high IRs in surveillance data from Finnmark. Corresponding gender ratios were observed in the high school based screenings in Philadelphia (girls 8.1% and boys 2.5%) [49], in New York (8.9% and 3.8%) [82], and in the first round of screening in New Orleans (11.5% and 6.2%) [81]. A similar gender ratio, but significantly lower prevalence was observed in the school based screenings in San Francisco (2.2% and 0.6%) [44], Luxembourg (2.5% and 0.9%) [48], and in a low-incidence area in Southern Norway (2.9% and 1.0%) [47] indicating that high school based screening may not be efficient use of resources in low-morbidity areas.

In our study, we confirmed that girls started to have sex at an earlier age than boys, more often were in steady relationships, had older partners, reported less time since last intercourse (results not shown) and were poorer condom users than boys at this occasion, which is in line with other Nordic studies on adolescent sexual behaviour [62-67, 126]. More boys than girls reported casual last sexual partners (21% vs 11%,  $p < 0.001$ ) and more alcohol use overall and related to last sexual intercourse, also in agreement with other studies [62, 63, 67, 126].

Accordingly, girls and boys had different risk profiles for infection.

Adolescent males reporting higher number of sexual partners than females is what particularly has separated genders in their responses in sexual behaviour surveys [62, 63, 81, 109]. In contrast, girls and boys in the FHSS reported similar number of sexual partners past 6 months. As girls had earlier sexual debut, more boys than girls reported 1-2 lifetime partners, while more girls than boys reported  $\geq 6$  lifetime partners. The results may indicate a new cohort of more sexually active and self-confident adolescent females as also suggested by others [67]. In contrast, recent studies in the same age group in Southern Europe found that adolescent

boys still report earlier sexual debut and higher lifetime number of partners than girls [55, 127, 128].

Among girls, several previously well-documented risk factors increased the odds of prevalent chlamydia infection in crude and multivariable analyses: ethnicity (Sami/Sami-Norwegian),  $\geq 2$  sexual partners past 6 months, and  $\geq 6$  lifetime partners [51, 112, 129]. In addition, higher maternal education and meeting venue for last partner increased odds of infection. We found that daughters of higher educated mothers reported more substance use overall and in connection with last intercourse than those with less educated mothers, which may suggest that higher educated women leave their daughters more freedom. The increased infection risk associated with particular meeting venues could reflect high-risk sexual behaviours and increased prevalence among persons frequenting these venues [130].

In boys, no condom use at first intercourse and having had an older last sexual partner increased the odds of infection in crude and multivariable analyses. Condom use at sexual debut was a strong predictor of condom use at last intercourse. Only 12% of the boys reported older last sexual partner, but this increased the odds of infection threefold in boys, which is similar to results from a recent study in the US [131]. This odds ratio became non-significant when adjusting for lifetime number of partners, indicating that adolescent boys who attract older women are more sexually active than peers with same-aged or younger partners.

In the multivariable model for girls and boys combined, residence outside the family home during school year,  $\geq 2$  partners past 6 months, meeting last partner at a party, bar or disco, and no condom use at last intercourse increased the odds of prevalent infection. One third of participants had left their home municipality to attend high school and they had twice the odds of infection compared to students living at home. The reasons may be less parental and societal control. A 1979 school survey on adolescent sexual behaviour and contraceptive use

in a Finnmark municipality found that girls living outside their family home were more sexually active than those living at home [66]. We found no other studies that have examined associations between residence during high school and prevalent chlamydia infection.

Due to detection of significantly more gender-specific genotypes (STs) in girls than boys, and most girls reporting older last sexual partners, we concluded that a majority of girls were linked to off-school sexual networks with assumed higher chlamydia rates as indicated by surveillance data (Paper I and II). Unlike what we expected, sexual partner age was not significantly associated with female chlamydia infection. The variable ‘partner age’ was based on age of participants’ last sexual partner. Girls reporting same-aged or younger sexual partners may have had recent older last partners, thus obscuring the association between partner age and infection risk in girls.

In Paper II, we showed that accumulation of gender-specific early sexual experiences may contribute to a different chlamydia risk profile in girls and boys. Nagelkerke’s estimate of explained variance in the multivariable models with outcome variable chlamydia infection was 13% in girls, 19% in boys, and 14% in both genders combined. We may have missed important behavioural chlamydia risk factors like concurrent sexual partnerships not assessed in the questionnaire [111-113]. We concluded that the genders are vulnerable to infections at different times during adolescence due to differing behaviour with girls on average initiating their sexual careers and being more sexually active at a younger age than boys. This suggests the need for gender-specific interventions in this age group.

#### ***5.3.4 Chlamydia testing in a high school based screening and previously in clinical practice***

In Paper III, we detected significant gender differences in previous clinic based testing as more than half of sexually active girls had been tested compared to only one-fifth of boys. This is in agreement with national surveillance data and previous studies in Norway and other

high-income countries having implemented extensive chlamydia testing or screening programmes [38, 43, 51, 79, 132].

The school based screening reached a high and similar proportion of both genders and proved efficient in terms of proportion of population tested, and number of infections detected and treated. The following factors were assumed to be important for the unusually high participation in the school based screening: thorough planning, the acceptance gained from the principals, the information provided to teachers, students and parents before data collection, the relevant topics, the universal offer to all students irrespective of sexual history, the ‘in-class’ recruitment and sampling procedures, the efficient logistics, rapid notification of positive test results, and FHSS being the first chlamydia high school based screening in Northern Norway [98, 133]. It is likely that invitation to participate in research increased uptake. External researcher led recruitment may also have contributed [134].

A high proportion of boys accepting the offer to be tested has also been observed in similar studies [47, 49, 82, 135]. Previous studies in non-clinical settings have found that easy access, convenient testing procedures, high levels of confidentiality and individual provider characteristics may influence boys’ decision to be tested [78, 80, 136-139].

Female participants in the FHSS had high chlamydia prevalence irrespective of previous clinic based testing; 7.3% if previous clinic based testing versus 7.2% if school-only test. Girls with clinic based testing had higher levels and longer duration of sexual risk behaviours than those with school-only test ( $p$ -values  $<0.03$  for the variables;  $\geq 2$  sexually active years,  $\geq 2$  partners past 6 months,  $\geq 6$  lifetime partners, older last partner, and no condom at last intercourse). The equal chlamydia prevalence may indicate effect of adherence to testing and treatment recommendations in girls with clinic based testing. This assumption is supported by the natural experiment that occurred in Sweden with nvCT that escaped detection and was

able to spread freely in mostly younger age groups [40, 120, 121]. Diagnosis, treatment and partner notification was discontinued in nvCT cases since they tested false negative. After correction of the diagnostic targets, a rebound chlamydia epidemic was observed (Figure 1) strongly indicating that early diagnosis and treatment does affect community transmission and decrease prevalence [120]. Alternatively, the two groups of girls had equal prevalence due to unrecognised risk factors in the school-only test group.

Boys with previous test had about the same prevalence as girls. The finding that boys with school-only test had less than half the prevalence of girls is consistent with less sexual activity in boys this age and hence reduced risk of infection (Paper II). Half of chlamydia infections were detected in the school-only test group, and correspondingly participants testing positive reported higher levels of sexual risk behaviours than participants with school-only test and negative test results.

High school based screening and previous clinic based testing were associated with completely different independent variables. In the multivariable analysis of girls and boys combined, known chlamydia risk factors such as female gender, young age at sexual debut, no condom use at first intercourse, and higher number of lifetime sexual partners increased the odds of clinic based testing. This indicates that these adolescents were aware of behavioural STI determinants. Among boys, testing varied by ethnic group which is in line with a recent Dutch study [79]. Nagelkerke's estimate of explained variance in the multivariable model for all participants with outcome 'clinic based testing' was 42% showing a good model fit, indicating that important variables were included in the model.

In contrast, several factors unknown to increase chlamydia infection risk in adolescents such as male gender, academic affiliation, later sexual debut, and condom use at first intercourse increased the odds of school based screening in the multivariable analysis of both genders

combined. The school based screening participants accepted test services they were not seeking. Based on the New Orleans school screening, Nsuami *et al.* have suggested that persons accepting school based STI screening are motivated by a collective acceptance of something that is being offered to everybody, rather than by a rationalisation of individual chlamydia risk [140].

Among all participants, 42% of girls and 7% of boys reported current urogenital symptoms, and this variable was observed to have the strongest association with school based screening in the multivariable analysis of both genders combined. However, 92% of participants without symptoms and 97% with symptoms were tested indicating high participation among those without urogenital complaints. A Nagelkerke's estimate of 6.2% may also reflect that accepting school based screening is not motivated by participants' sexual risk profile. Boys tested in the FHSS frequently commented on the convenience of 'everybody getting tested', which also has been emphasised by male participants in other non-clinical screening studies [80]. Engaging boys in chlamydia testing early on may constitute a preventive strategy for their female partners, normalise testing, and increase young men's interest in ensuring their own sexual health [111].

High school students represent easily accessible populations. Repeat annual screening and treatment with high enough participation should theoretically have the potential to reduce the transmission and reservoir of chlamydia infections in the target population [3, 141]. However, participation in the repeat high school based screening programme in New Orleans declined from an initial 56% in 1995-96 down to 33% in the final year 2004-05 [135]. This was mainly due to decrease in number of students with parental consent. Only a limited number of students were enrolled for multiple years and sexual behaviour was recorded only for a few years. In New Orleans, repeat screening was not associated with significant change in

chlamydia prevalence in females or males among those who were tested more than once [142]. In Philadelphia, there was only a slight decline in female positivity from 8.4% in the first year to 7.2% in year 5, while male positivity remained at 2.5% [143]. The failure to reduce prevalence has been explained by incomplete coverage, links to off-school sexual networks with higher infection prevalence, inability to reach high-risk core group members, inadequate partner notification and treatment, and insufficient screening frequency [141].

Repeat high school based chlamydia screening has not been tried in Norway, but repeat high school studies on adolescent health and lifestyle including biological samples have shown sustained response rates above 85% [133, 144]. In addition, parental consent only being required in participants <16 years, most students attending three consecutive school years, the homogeneous nature of the Norwegian society, the liberated attitudes towards adolescent sexuality, and the high participation rate in the FHSS may suggest a potential for sustained higher participation in repeat school based screenings in Norway compared to the US [135].

In the past years, the evidence that asymptomatic lower genital chlamydia infection is likely to cause pelvic inflammatory disease and reproductive complications has been questioned [11, 145-147] and consequently the evidence that supports large screening programmes in the population [73, 148-150]. Given the high participation rate in the FHSS that provided access to almost entire birth cohorts of adolescents, repeat high school based studies on changing trends in sexual behaviour using chlamydia infection as a biomarker for risk behaviour may still be valuable. The results could be used to develop innovative targeted interventions to increase safe sexual behaviour among adolescents. We thus suggest conducting repeat high school based screenings in selected high-morbidity areas designed as research studies with continuous evaluation of feasibility, cost, participation, and effect on prevalence [151].

## 6. Conclusions

In summary, our findings suggest that:

- MLST of *C. trachomatis* had significantly higher resolution than traditional *ompA* genotyping and enabled the detection of specific STs such as the Swedish nvCT. We found multiple novel alleles, new STs, and unique STs in line with studies on different bacteria in previously unmapped geographic areas and in accordance with other newly established bacterial MLST databases. There were no significant differences in genetic diversity of STs between the three areas. Due to high resolution and detection of specific STs, we concluded that MLST is a useful tool in molecular chlamydia epidemiology.
- The high chlamydia prevalence of 5.7% detected in the FHSS corresponded to the high annual IR in surveillance data observed in Finnmark. Gender differences in sexual behaviour in the early sexually active years contributed to gender differences in risk profiles for chlamydia infection. This probably contributed to girls having twice the prevalence of boys. Girls and boys being vulnerable to chlamydia infections at different times during adolescence due to behavioural factors suggests the need for gender-specific chlamydia control strategies in this age group.
- Threefold more adolescent girls than boys reported previous clinic based chlamydia testing. Previous testing was associated with mostly known chlamydia risk factors suggesting awareness of behavioural determinants. An unusually high and equal proportion (93%) of sexually active girls and boys were tested in the school based screening, which was mostly associated with factors unknown to increase infection risk. Girls had high and equal prevalence independent of previous testing. Half of chlamydia infections were detected in participants never tested. The high participation



and detection and treatment of a large chlamydia reservoir suggests school based screening as a potential tool to decrease chlamydia transmission among sexually active adolescents in high-morbidity areas in Norway.

## **7. Implications for future research**

The findings in this thesis related to genotyping, participation, chlamydia prevalence, sexual behaviour, and testing patterns suggest several interesting questions to be further explored.

The FHSS sexual behaviour data (sexual debut age, circumstances related to first intercourse, attitudes and feelings towards sex among those without sexual debut) will be further explored in separate papers, and so will the associations between chlamydia infections and self-assessed risk, condom use, and sexual behaviour on the Internet.

When possible, WGS should be applied to chlamydia samples collected in a general population. WGS data should be linked to questionnaire data on urogenital symptoms, sexual behaviour, and sexual networks. The unique ST distribution in Finnmark should be further examined in a repeat study including a larger study population and more chlamydia samples. WGS should in particular be applied to ST161/genovar G chlamydia samples.

In future studies, self-collected vaginal swabs rather than FVUs should be used to increase sensitivity in the female samples. The sexual behaviour data should be more comprehensive and include different sexual practices (vaginal, oral and anal intercourse), concurrent relationships, and sexual partner characteristics such as school enrolment, ethnicity, country of origin, and risk assessment of partner. Sexual partners should be included in the study for prevalence detection and genotyping of positive specimens. Previous clinic based testing should be validated using laboratory data. Partner treatment rates should be assessed. High schools in other high-morbidity areas in Norway should be included to increase generalisability. Repeat high school based screening studies including comprehensive sexual behaviour questionnaires in selected high-morbidity areas should be tried and evaluated.

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# Paper I



# Multilocus Sequence Typing of Genital *Chlamydia trachomatis* in Norway Reveals Multiple New Sequence Types and a Large Genetic Diversity

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## Abstract

**Background:** The *Chlamydia trachomatis* incidence rate in Finnmark, the most northern and sparsely populated county in Norway, has been twice the national average. This population based cross-sectional study among Finnmark high school students had the following aims: i) to examine distribution of multilocus sequence types (STs) of *C. trachomatis* in a previously unmapped area, ii) to compare chlamydia genetic diversity in Finnmark with that of two urban regions, and iii) to compare discriminatory capacity of multilocus sequence typing (MLST) with conventional *ompA* sequencing in a large number of chlamydia specimens.

**Methodology:** *ompA* sequencing and a high-resolution MLST system based on PCR amplification and DNA sequencing of five highly variable genetic regions were used. Eighty chlamydia specimens from adolescents aged 15–20 years in Finnmark were collected in five high schools (n=60) and from routine clinical samples in the laboratory (n=20). These were compared to routine clinical samples from adolescents in Tromsø (n=80) and Trondheim (n=88), capitals of North and Central Norway, respectively.

**Principal Findings:** *ompA* sequencing detected 11 genotypes in 248 specimens from all three areas. MLST displayed 50 STs providing a five-fold higher resolution. Two-thirds of all STs were novel. The common *ompA* E/Bour genotype comprised 46% and resolved into 24 different STs. MLST identified the Swedish new variant of *C. trachomatis* not discriminated by *ompA* sequencing. Simpson's discriminatory index (*D*) was 0.93 for MLST, while a corrected *D<sub>c</sub>* was 0.97. There were no statistically significant differences in ST genetic diversity between geographic areas. Finnmark had an atypical genovar distribution with G being predominant. This was mainly due to expansion of specific STs of which the novel ST161 was unique for Finnmark.

**Conclusions/Significance:** MLST revealed multiple new STs and a larger genetic diversity in comparison to *ompA* sequencing and proved to be a useful tool in molecular epidemiology of chlamydia infections.

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## Introduction

Despite widespread efforts to control *Chlamydia trachomatis*, it remains the leading cause of bacterial sexually transmitted infections in Scandinavia and worldwide. The prevalence is highest among 15–24 year-olds [1]. In Norway, genital chlamydia infections have been part of the national surveillance system for communicable diseases since 2003. Treatment is free of charge, and partner tracing is compulsory. As in other western countries

having implemented extensive chlamydia testing, the reported number of chlamydia infections in Norway almost doubled since the mid-1990s [1]. The highest incidence rates have been reported in Finnmark, the most northern and sparsely populated county in Norway with an incidence rate of 8.98/1000 in 2009, almost twice the national average (4.67/1000) [2].

Strain typing of *C. trachomatis* is important to understand the genetic population structure and is a useful tool in epidemiological studies, in investigation of infection transmission or recurrence, in

sexual network analysis, and in surveillance of emerging strains such as the Swedish new variant of *C. trachomatis* (nvCT) [3]. It is assumed that persons infected by the same chlamydia strain are more likely to be epidemiologically linked than those infected with different strains. Traditional typing differentiated genital *C. trachomatis* into subgroups based on serospecificity for the major outer membrane protein (MOMP), encoded by the *ompA* gene. MOMP and *ompA* based methods have predominated typing in the past decades [4] where sequencing of the *ompA* gene has provided the best discriminatory capacity [5]. As these methods identified only a limited number of distinct subtypes, and the various subtypes could persist for a long time within a geographic area, research has focused on developing strain typing techniques with higher capacity of resolution. Several alternative typing systems for *C. trachomatis* have been published in recent years. Two standard multilocus sequence typing (MLST) approaches based on housekeeping genes have a discriminatory capacity comparable to *ompA* and could be useful for slowly evolving processes in evolutionary studies, but were not used in this study due to limited resolution [6,7]. A significantly higher resolution has been shown for a multilocus variable number of tandem repeats (VNTR) analysis (MLVA) system [8,9], but in an evaluation it was found that some VNTR markers may vary with replication of single clones and cause difficulties in interpretation [10]. In our study, we used the MLST system developed by Klint et al. for *C. trachomatis* based on PCR amplification and DNA sequencing of five highly variable target regions (not house keeping genes), that has displayed a three-fold higher resolution than *ompA* sequencing [11] and with a resolution similar to MLVA [12]. The target stability of this MLST scheme has proved satisfactory through sequencing studies of the nvCT [3,13,14] and of lymphogranuloma venereum *C. trachomatis* strains [15]. The scheme has been applied in several Swedish studies [11,13], and the multilocus sequence types (STs) have been included in the Uppsala University *C. trachomatis* MLST database (<http://mlstdb.bmc.uu.se>) enabling us to compare STs sampled in our study to STs collected in Sweden. We expected to find a proportion of common *C. trachomatis* STs in neighbouring countries Norway and Sweden, including the nvCT.

The aims of our study were: i) to examine distribution of *C. trachomatis* STs in an adolescent population in an unmapped high-incidence area in North Norway, ii) to compare the genetic diversity in a remote sparsely populated county with that of two urban regions in Norway, and iii) to compare the discriminatory capacity of the MLST scheme developed by Klint et al. with conventional *ompA* sequencing by applying both methods to a large number of chlamydia specimens from different geographic locations. To achieve this, we conducted a population based cross-sectional study collecting chlamydia specimens from high school students in Finnmark county, an extended county with minor municipalities and a population of only 72,500 ([www.ssb.no](http://www.ssb.no), Statistics Norway). Additional chlamydia specimens from adolescent girls and boys were collected from routine clinical samples in Tromsø and Trondheim, capitals of North and Central Norway, respectively. Our approach resulted in a total of 248 *C. trachomatis* specimens that were successfully genotyped, enabling us to assess genetic diversity within the different catchment areas, and compare the resolution of the two methods.

## Methods

### Study population and urine sampling

A population based cross-sectional study was conducted among girls and boys in five senior high schools in Finnmark county

during fall 2009 (manuscript in preparation). Briefly, the participants filled in a web-based questionnaire on demography, sexual behaviour and urogenital symptoms, and provided first-void urine samples under supervision of the study staff, giving a total of 60 chlamydia specimens from 1,476 urine samples that were analysed at the laboratory at the University Hospital of North Norway (UNN, Tromsø). Parallel to the high school study, 20 and 80 chlamydia positive urine samples from 15–20 year-olds in Finnmark and the Tromsø region, respectively, were consecutively collected from routine clinical samples at UNN Tromsø. Eighty-eight samples from patients of the same age group in the Trondheim region were collected at St. Olavs Hospital (Central Norway). After processing, a total of 248 chlamydia samples were immediately frozen at  $-70^{\circ}\text{C}$  in the laboratories and later transported on dry ice to the University Hospital of Uppsala (Sweden) for genotyping.

### Laboratory testing of urine samples

**Chlamydia PCR.** The UNN laboratory extracted DNA using the BUGS'n BEADS TM-STI kit (NorDiag ASA, Oslo, Norway) and used the ProCt real-time PCR (ProCelo A/S, Tromsø, Norway) with sensitivity 97% and specificity 100%. The Trondheim laboratory prepared DNA using the bacterial protocol on GenoM 48 (Qjagen, Hilden, Germany) and used an in-house triplex real-time PCR (cryptic plasmid, MOMP gene and internal control) with sensitivity 96% and specificity 100% [16]. A plasmid specific PCR was used to confirm MLST identification of the nvCT [17].

**Strain typing.** *ompA* sequence determination was performed according to a previously described method [18] and strains were categorized into genovars D–K and *ompA* genotypes. Genovars denote subgroups of *C. trachomatis* based on serospecificity for MOMP inferred from *ompA* sequencing. Genotypes are subgroups based on *ompA* sequencing. The MLST scheme comprises five highly variable target regions and was performed as previously described [11] except that the *pbpB* region was amplified as two separate fragments according to Jurstrand et al. [13]. Allele numbers were assigned by comparing the sequence at each locus to all known corresponding alleles available in the Uppsala University *C. trachomatis* MLST database (<http://mlstdb.bmc.uu.se>). Allele profiles based on the five genetic regions are expressed as multilocus sequence types (STs). At baseline date February 16<sup>th</sup> 2010, the database included 145 STs originating from 467 chlamydia isolates. In our study, clonal complexes are defined as clusters of genetically related STs with only one allele difference, i.e. single-locus variants (SLVs). The founder of a clonal complex is the ST that differs from the largest number of other STs at only a single locus, i.e. the ST that has the highest number of SLVs.

### Ethics

In the high school study, written informed consent was obtained from the next of kin, carers or guardians on the behalf of participants younger than 16 years. Participants 16 years or older gave their informed consent by filling in a web-based questionnaire in accordance with the Health Research Act §17.b stating their right to consent. All procedures were approved by the Regional Committees for Medical and Health Research Ethics North Norway (REK Nord No.: 200900528-6/MRO/400) and the Data Protection Officer at UNN (No.: 2009/2475). Establishment of a research bio-bank for *C. trachomatis* specimens was approved by The National Directorate of Health (Bio-bank Registry No. 2723).

**Table 1.** Number of genetic variants of *ompA* sequencing and multilocus sequence typing (MLST) within each genovar (D–K) in 248 *C. trachomatis* specimens.

Chlamydia genovars			Number of genetic variants		
Genovar	No of specimens	(%)	<i>ompA</i> genotypes	STs <sup>1</sup>	STs with <i>ompA</i>
D	21	(8.5)	2	3	3
E	115	(46.4)	2	24	25
F	44	(17.7)	1	6	6
G	47	(19.0)	2	9	9
H	5	(2.0)	1	2	2
I	1	(0.4)	1	1	1
J	1	(0.4)	1	1	1
K	14	(5.6)	1	6	6
Total	248	(100.0)	11	50 <sup>2</sup>	53 <sup>2</sup>

<sup>1</sup>Sequence types (STs) of *C. trachomatis* detected by MLST.

<sup>2</sup>The numbers reflect the total number of unique genetic variants in the 248 chlamydia specimens and do not equal the sum of each column.

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## Statistical methods

SPSS 18.0 for Windows was used for statistical analysis of the associations between urogenital symptoms, and STs and clonal complexes (chi-square). Binominal confidence intervals were calculated according to Clopper-Pearson exact method [19]. The discriminatory power ( $D$ ) of a typing method refers to the probability that two unrelated strains sampled from the test population will be placed into different typing groups.  $D$  was determined for *ompA* genotyping and MLST in the 188 routine clinical samples using Hunter and Gaston's modification of Simpson's discriminatory index [20]:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^S n_j(n_j - 1)$$

where  $N$  is the number of unrelated strains tested,  $s$  is the number of different types, and  $n_j$  is the number of strains belonging to the  $j$ th type. Confidence interval (CI) for  $D$  was calculated as originally described by Simpson [21]. A cut-off value for  $D$  of  $\geq 0.95$  for a molecular typing method is considered 'ideal' [22]. As the 188 samples were consecutively collected in the laboratories from a defined age group and within a limited time frame from defined geographic areas, a degree of epidemiological relatedness could not be excluded. The following assumptions were made: the two most common STs in an 'ideal' epidemiologically independent sample will have prevalences equal to the third most prevalent ST. Thus,  $n_{ST12}$  and  $n_{ST56}$  were set equal to  $n_{ST153}$  ( $n = 15$ ), and a corrected  $D_c$  was calculated. BioNumerics software (version 6.01, Applied Maths, Sint-Martens-Latem, Belgium) was used to generate a minimum spanning tree (under the categorical coefficient of similarity and the priority rule of the highest number of single-locus variants).

## Results

A complete MLST profile was obtained for all 248 chlamydia specimens identifying a total of 50 STs (Table 1). *ompA* sequencing detected 11 genotypes, thus the MLST scheme provided 4.5 higher resolution than *ompA*. By combining MLST and *ompA*, 53 unique genotypes were identified. The commonly predominating *ompA* E/Bour genotype comprised 46% of all specimens and could

be further resolved by the MLST system into 24 different STs, i.e. giving 24 times higher resolution. Nineteen percent of all specimens belonged to genovar G which could be further resolved into nine different STs.

Simpson's discriminatory index ( $D$ ) was calculated in the 188 routine clinical samples (Table S1) and was 0.93 (95% CI 0.91–0.95) for MLST and 0.67 (95% CI 0.61–0.73) for *ompA* sequencing, respectively. A corrected  $D_c$  of 0.97 (95% CI 0.96–0.98) was calculated for MLST.

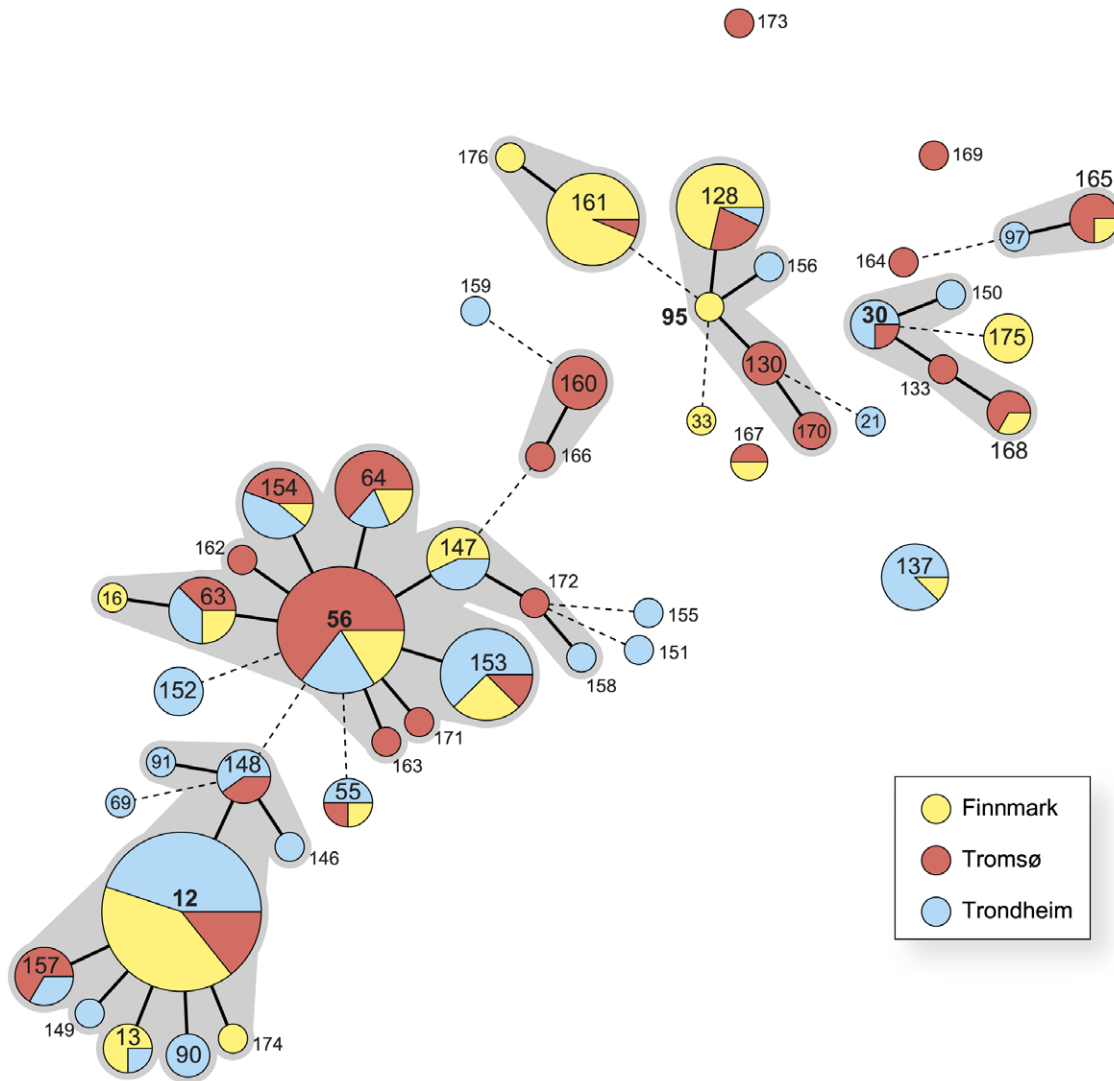
Among the 50 STs, 31 STs (62%) were novel, while 19 STs had been identified previously. Novel STs were numbered in order of identification: ST146–ST176 (Table S2). Four of the 50 STs were singletons, i.e. differing at more than two alleles from all other isolates. Fifty-two percent of the STs comprised only one specimen and 62% had less than four specimens.

A total of 12 new alleles in the MLST scheme were detected comprising 9% of all specimens (Table S2). The three most variable regions, *pbpB*, *hctB* and CT058 displayed five, three and three new alleles, respectively. The less variable regions CT144 had one new allele and CT172 had none. Most of the new alleles were substitutions of a single base pair.

All 248 chlamydia specimens were clustered using a minimum spanning tree based on the STs (Figure 1). ST12, ST30, ST56, and ST95 were considered putative founders of a clonal complex. All four were present in the MLST database prior to our study. ST12 (20%) and ST56 (13%) were also the most frequent clones and were present at all three collection sites. Of all specimens, 57% (142 of 248) belonged to STs present in all three areas, and included eight STs, of which ST153 and ST154 were new. Sixty-four percent (32 of 50) of the STs were unique for specific areas. Differences in genetic diversity as estimated by ST variation and proportion of novel STs were not statistically significant between the three geographic areas (Table 2).

Four of the 248 specimens were identified as ST55 which appears to be unique to the new Swedish variant of *C. trachomatis* (nvCT) [17]. One nvCT specimen was found in Finnmark and Tromsø, respectively, as were two in Trondheim.

Genovar E was most prevalent in both Tromsø (65%, 95% CI 54–75%) and Trondheim (48%, 95% CI 37–59%) and significantly more frequent than in Finnmark (26%, 95% CI 17–36%). Finnmark had the most atypical genovar distribution with G being predominant, contributing 36% (95% CI 26–47%), which was



**Figure 1. Minimum spanning tree analysis based on 50 multilocus sequence types (STs) in 248 chlamydia specimens.** The 50 circles correspond to different STs discriminated by multilocus sequence typing (MLST). Each circle represents an ST, and ST number is given inside or next to the circle. Circle size reflects the number of isolates. Bold black lines connect single-locus variants (SLV). Broken lines connecting double-locus variants are only indicative as several alternative links with equal weight may exist. The coloured pie charts indicate ST geographic distribution. Grey shaded areas define clonal complexes i.e. clusters of genetically related STs with only one allele difference.  
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significantly higher than in Tromsø (13%, 95% CI 6–22%) and Trondheim (9%, 95% CI 4–17%). The predominance of genovar G in Finnmark was mainly due to the expansion of ST128 and the novel ST161 (Figure 1).

In Finnmark, ST12 (25%), ST161 (19%) and ST128 (13%) were the most prevalent chlamydia clones (Figure 1). The novel ST161 was almost unique for Finnmark. It was identified in four of the five high schools and was equally distributed between the genders. In subjects infected with ST161, there were as many symptomatic as asymptomatic individuals. ST128 was not prevalent in Tromsø (3%) or Trondheim (1%).

Among the 20 STs identified in the Finnmark high school study, six STs were found in both genders, twelve STs were present in girls only, and two STs were present only in boys. The founders ST12 and ST56, and the novel ST161, were among the six STs shared between genders. Among the two STs found in boys only, one specimen of ST33 was identified in a male participant in Finnmark who reported having sex with men. Chlamydia infected

girls had a higher proportion of samples with gender-specific STs (34%, 95% CI 20–51%) compared to infected boys (11%, 95% CI 1.3–33%).

Among participants in the high school study, 59% of chlamydia infected girls and 22% of infected boys ( $p=0.01$ ) reported urogenital symptoms. No statistically significant associations between clinical symptoms and specific STs or clonal complexes were found.

## Discussion

This is the largest study to date using this MLST system and is also the study where MLST has outperformed *ompA* the most by offering a five-fold higher resolution than *ompA* genotyping, compared with the three-fold increase described earlier [11]. We observed a discriminatory index  $D$  of 0.93 (95% CI 0.91–0.95) which was slightly lower than expected in such a large number of samples. A cut-off value  $\geq 0.95$  is considered 'ideal' for molecular

**Table 2.** Geographic distribution of *C. trachomatis* specimens according to three different strain typing methods, and genetic diversity within each area.

Geographic area	Finnmark <sup>1</sup>	Tromsø	Trondheim	Total <sup>2</sup>
No of chlamydia specimens	80	80	88	248
No of STs <sup>3</sup>	21	27	28	50
No of novel STs	10	18	14	31
No of cases with novel STs	33	34	34	101
ST variation <sup>4</sup> (95% CI)	0.26 (0.17–0.40)	0.34 (0.23–0.44)	0.32 (0.22–0.41)	0.20 (0.15–0.25)
% novel STs <sup>5</sup> (95% CI)	47.6 (26.3–69.0)	66.7 (48.9–84.5)	50.0 (31.5–68.5)	62.0 (48.6–75.5)
No of genovars <sup>6</sup> D–K	6	7	7	8
No of <i>ompA</i> genotypes <sup>7</sup>	7	10	9	11

CI: Confidence interval.

<sup>1</sup>*C. trachomatis* specimens from either the high school study (n = 60) or routine clinical samples in the laboratory (n = 20).

<sup>2</sup>The numbers reflect the results for all 248 specimens and do not necessarily equal the sum of each row.

<sup>3</sup>Sequence types of *C. trachomatis* detected by multilocus sequence typing.

<sup>4</sup>Number of STs identified in an area divided by number of chlamydia specimens in the area.

<sup>5</sup>Percentage novel STs in an area of total number of STs in the area.

<sup>6</sup>Genovar D–K of *C. trachomatis* inferred from *ompA* sequencing.

<sup>7</sup>Genotypes of *C. trachomatis* detected by *ompA* sequencing.

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typing methods [22]. The high prevalence of ST12 and ST56 could indicate that the 188 laboratory samples were not completely epidemiologically independent, and we therefore decided to use a prevalence correction for the two most frequent STs. We calculated a significantly higher corrected  $D_c$  of 0.97 (95% CI 0.96–0.98) with the entire confidence interval above the cut-off value. Two previous studies fulfilling the above sampling criteria, but with only a small number of samples (both n = 31) reported  $D$  between 0.95 and 0.96 for this MLST scheme [10,12]. Confidence intervals were not assessed in these studies. As  $D$  includes no correcting factor for small populations, typing schemes should not be validated with small samples [20].

The MLST scheme resolved the chlamydia specimens into a number of STs of which a significant proportion comprised only a few specimens and two-thirds were novel. The minimum spanning tree analysis (Figure 1) showed that the majority of specimens belonged to clonal complexes which have also been observed in other bacterial MLST databases [23]. Organization into clonal complexes makes MLST data more suitable to epidemiologic analysis and reduces the potential of over-discrimination. The multiple novel STs could be due to the relatively short existence of the database only since 2007. In addition, genotyping of chlamydia strains from individuals in an unmapped geographic area will commonly identify a number of novel STs. As the database expands with time, it is expected that genetic relationships between more STs will be revealed. Prior to this study, Norwegian chlamydia specimens from heterosexuals had not been characterized using this MLST scheme.

Among the founders of clonal complexes, ST12 was the most prevalent constituting one-fifth of the strains in all three areas. ST12 is common among both heterosexuals and men having sex with men (MSM) in Sweden and other European countries. ST30 and ST56 are also frequently reported to the database. The founder ST95 (one female, Finnmark) had previously been identified in only three samples from Dutch females illustrating how an individual through sexual contact might have interconnected geographically distant areas.

A nvCT prevalence of 1.6% was as expected as nvCT has rarely been identified outside Sweden [24]. These infections could have been imported directly from Sweden, but may also reflect

domestic spread. As the questionnaire did not include ethnicity or origin country of former sex partners, we could not examine any links to Sweden. A previous study found that the nvCT prevalence in Oslo increased from 1.0% in the first quarter of 2007 to 3.4% in the second quarter of 2008, indicating a slow spread within Norway [25]. The laboratories in Tromsø and Trondheim have used nvCT sensitive diagnostic assays since 2005 and 2006, respectively, implying that the nvCT clone has not escaped detection in these areas.

One specimen from Finnmark contained an ST33 genotype which had previously only been found among MSM in Stockholm (Sweden) and France. ST33 was detected in a Finnmark male who reported having sex with men which could indicate links to international MSM networks. Due to limited epidemiological data on previous sex partners we could not confirm this hypothesis. The discrimination of nvCT and ST33 is not possible using *ompA* sequencing.

Genovar E was the most common genovar in Tromsø and Trondheim, as in heterosexual populations elsewhere [5,26]. The predominance of genovar G in Finnmark is unusual in heterosexual populations and was mainly due to the expansion of ST128 and ST161. As the 20 routine clinical samples also were restricted to the 15–20 year-olds, we could not determine whether the genovar distribution in our study reflects the distribution in the general population in Finnmark. The uniquely high occurrence of ST128 and ST161 in Finnmark and no significant spread to neighbouring Tromsø may be explained by these clones being limited to local sexual networks in Finnmark. However, we could not confirm this hypothesis due to lack of sexual network information.

Possible factors contributing to the success of ST12, ST128 and ST161 in Finnmark could be a high transmission rate reflecting increased tissue tropism, or the strains causing a silent infection escaping discovery. However, all three strains were symptomatic in approximately half of infected participants in the high school study. Due to only 60 chlamydia specimens carrying behavioural data, the reasons for the success of ST12, ST128 and ST161 cannot be further elucidated. As previously shown, no associations between urogenital symptoms and specific STs or clonal complexes were found [27].

The chlamydia infected girls in the high school study had a higher proportion of gender-specific STs compared to boys. This may indicate that a significant proportion of female students had off-school sex partners, and therefore were infected with STs not identified in their high school male peers. This was supported by the girls reporting older partners at last intercourse (19.9 years) compared to the boys (16.3 years,  $p < 0.01$ ). The propensity of young girls to have older partners has also been shown in other studies [28].

The achievement of a complete MLST profile for all 248 samples was unexpected compared to previous studies. However, all specimens were new and fresh, they were frozen at  $-70^{\circ}\text{C}$  immediately after the first diagnostic PCR, and they were thawed for the first time prior to MLST to avoid degradation of DNA. In addition, the MLST method has been optimized since the introduction in 2007 which also could have contributed to the high success rate [13]. Thus, we consider the results reliable.

Presently our MLST system is too labour intensive to enable epidemiological analysis in clinical routine with partner notification. Future research should focus on development of a typing scheme with a high discriminatory power that allows for rapid and easy interpretation, but which also is economically affordable. Next generation sequencing technologies may in the future reach this objective. In an area where the chlamydia STs are known, array-based methods for analysis of sequence variation might be an alternative, but this approach will not detect STs with novel alleles [29].

In conclusion, our study shows that this MLST scheme is a valuable tool for studying the molecular epidemiology of *C. trachomatis* infections and far superior to *ompA* typing in terms of resolution especially of the globally predominant genovar E.

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## Supporting Information

**Table S1 188 *C. trachomatis* specimens from routine clinical samples in the laboratories resolving into 46 multilocus sequence types (STs) listed by: ST number, the corresponding *ompA* genotype and genovar (D–K), and number of specimens within each ST.**

(DOC)

**Table S2 248 *C. trachomatis* specimens resolving into 50 multilocus sequence types (STs) listed by: ST number, the five specific alleles making up the MLST profile, the corresponding *ompA* genotype and genovar (D–K), and number of specimens within each ST.**

(DOC)

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## Author Contributions

Conceived and designed the experiments: KG BH ASF GSS. Performed the experiments: LC AS KØ. Analyzed the data: KG LC BH. Contributed reagents/materials/analysis tools: BH KG. Wrote the paper: KG LC BH GSS ASF AS KØ. Data collection in The Finnmark High School Study: KG. Applications for financing: KG.

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Table S1. 188 *C. trachomatis* specimens from routine clinical samples in the laboratories, resolving into 46 multilocus sequence types (STs) listed by: ST number, the corresponding *ompA* genotype and genovar type (D-K), and number of specimens within each ST.

ST	<i>ompA</i> genotype	Genovar D-K	No of specim.
12	1, 24, 6	D, F, E	35
13	24	F	2
21	2	D	1
30	12	K	4
55 <sup>1</sup>	6	E	3
56	6	E	28
63	6	E	6
64	6, 7	E	10
69	6	E	1
90	24	F	3
91	24	F	1
95	11	G	1
97	35	H	1
128	11, 41	G	5
130	11	G	3
133	12	K	1
137	11	G	7
146	6	E	1
147	6	E	5
148	24	F	5
149	24	F	1
150	12	K	1
151	6	E	1
152	6	E	4
153	6	E	15
154	6	E	8
155	6	E	1
156	36	I	1
157	6	E	6
158	6	E	1
159	6	E	1
160	6	E	5
161	11	G	3
162	6	E	1
163	6	E	1
164	11	G	1
165	35	H	3
166	6	E	1
167	2	D	1
168	12	K	2
169	20	J	1
170	11	G	2
171	6	E	1
172	6	E	1
173	12	K	1
175	12	K	1
n=46	n=11	n=8	n=188

<sup>1</sup>new Swedish mutant of *C. trachomatis*

Table S2. 248 *C. trachomatis* specimens resolving into 50 multilocus sequence types (STs) listed by: ST number, the five specific alleles making up the MLST profile, the corresponding *ompA* genotype and genovar (D-K), and number of specimens within each ST.

ST	MLST profile					<i>ompA</i> genotype	Genovar D-K	No of specim.
	<i>hctB</i>	CT058	CT144	CT172	<i>pbpB</i>			
12	5	19	7	1	4	1, 24, 6	D, F, E	49
13	5	19	15	1	4	24	F	4
16	7	19	14	2	1	6	E	1
21	10	4	1	4	23	2	D	1
30	10	7	9	3	8	12	K	4
33	10	8	5	3	6	11	G	1
55 <sup>1</sup>	21	19	1	2	1	6	E	4
56	1	19	7	2	1	6	E	31
63	7	19	7	2	1	6	E	8
64	4	19	7	2	1	6, 7	E	11
69	5	19	6	2	2	6	E	1
90	5	19	1	1	4	24	F	3
91	5	19	5	2	4	24	F	1
95	10	8	1	3	5	11	G	1
97	12	12	11	9	21	35	H	1
128	10	8	1	4	5	11, 41	G	14
130	10	4	1	3	5	11	G	3
133	10	7	9	1	8	12	K	1
137	10	36	22	4	6	11	G	8
146	5	19	7	2	47*	6	E	1
147	1	19	7	2	2	6	E	7
148	5	19	7	2	4	24	F	5
149	5	19	7	1	48*	24	F	1
150	10	7	9	3	49*	12	K	1
151	1	2	6	2	50*	6	E	1
152	47*	38*	7	2	1	6	E	4
153	35	19	7	2	1	6	E	16
154	48*	19	7	2	1	6	E	9
155	12	2	6	2	2	6	E	1
156	10	8	1	3	18	36	I	1
157	5	19	7	1	1	6	E	6
158	5	2	7	2	2	6	E	1
159	35	2	1	1	2	6	E	1
160	10	2	1	2	2	6	E	5
161	8	5	1	3	5	11	G	16
162	1	19	7	2	51*	6	E	1
163	1	39*	7	2	1	6	E	1
164	12	12	25*	9	5	11	G	1
165	12	12	9	9	21	35	H	4
166	10	19	1	2	2	6	E	1
167	12	15	1	3	23	2	D	2
168	10	7	9	1	13	12	K	3
169	29	5	12	4	18	20	J	1
170	10	4	1	3	6	11	G	2
171	49*	19	7	2	1	6	E	1
172	1	2	7	2	2	6	E	1
173	10	40*	16	4	21	12	K	1
174	5	19	7	1	2	6	E	1
175	10	6	22	3	8	12	K	4
176	8	5	5	3	5	11	G	1
n=50	n=13	n=13	n=12	n=5	n=15	n=11	n=8	n=248

\*novel allele, <sup>1</sup>new Swedish mutant of *C. trachomatis*



# Paper II



RESEARCH ARTICLE

Open Access

# Early sexual behaviour and *Chlamydia trachomatis* infection – a population based cross-sectional study on gender differences among adolescents in Norway

Kirsten Gravningen<sup>1,2\*</sup>, Anne-Sofie Furberg<sup>1,2</sup>, Gunnar Skov Simonsen<sup>1,3</sup> and Tom Wilsgaard<sup>2</sup>

## Abstract

**Background:** Early sexual behaviour has been shown to differ significantly between genders, but few studies have addressed this topic to explain the commonly observed differences in chlamydia rates between adolescent girls and boys. Our study aimed to determine chlamydia prevalence in adolescents aged 15–20 years in a high-incidence area in Norway, and to identify gender-specific early sexual behaviours associated with infection.

**Methods:** A population based cross-sectional study was conducted among all high school students in five towns in Finnmark county in 2009, using a web-based questionnaire and real-time *Chlamydia trachomatis* PCR in first-void urine samples (participation rate 85%, 800 girls/818 boys, mean age 17.2 years). Crude and multivariable logistic regression models were applied with chlamydia test result as dependent variable.

**Results:** Prevalence of chlamydia infection was 5.7% (95% confidence interval, CI, 4.4–7.3%). Girls were twice as likely to be infected as boys (7.3%, 5.3–9.7 vs 3.9%, 2.3–6.0). Girls reported earlier sexual debut, older partners, higher lifetime number of partners, and were poorer condom users. In girls, higher maternal education (odds ratio, OR, 2.2, 95% CI 1.1–4.4),  $\geq 2$  sexual partners past 6 months (OR 3.6, 1.8–7.3), and partner meeting venue at a private party, bar or disco (OR 5.0, 1.1–22.7) increased the odds of infection in the multivariable model. In boys, condom use at first intercourse (OR 0.06, 0.01–0.42) decreased the odds of infection, while having an older last sexual partner (OR 3.7, 1.3–11.0) increased the odds. In all participants, the risk of infection increased if residence outside the family home during school year (OR 2.0, 1.2–3.6), and decreased if condom was used at last intercourse (OR 0.2, 0.1–0.8).

**Conclusions:** We detected significant gender differences in chlamydia prevalence and sexual behaviours, and accordingly differing independent risk factors for chlamydia infection. We suggest that accumulation of essentially different experiences in the early sexually active years contribute to gender disparities in chlamydia risk in individuals this age. Gender-specific approaches may be the best alternative to control chlamydia infection in age group 15–20 years.

**Keywords:** Chlamydia trachomatis, Adolescent, Sexual behaviour, Gender differences, Cross-sectional study

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## Background

*Chlamydia trachomatis* is the most commonly reported sexually transmitted infection (STI) in Europe and USA mainly affecting young individuals aged 15–24 years, and is more often diagnosed in adolescent females than in males [1,2]. The true incidence of chlamydia in both genders is assumed to be higher than the reported numbers as the majority of infections are asymptomatic [3].

Early heterosexual behaviour has been shown to differ significantly between genders in the Nordic countries [4], but few studies have addressed this topic to explain the commonly observed differences in chlamydia rates between adolescent girls and boys using biological samples. The higher chlamydia incidence rates among female adolescents in surveillance data has commonly been linked to more extensive testing of girls due to their health seeking behaviour and the fact that screening strategies and reproductive health services mainly target females [1,5]. However, several population based studies have also detected significantly higher chlamydia prevalences in adolescent girls than in same-aged boys [6-9]. This has mainly been attributed to girls being biologically more susceptible to chlamydia infections than boys, and also to increased exposure due to social and cultural factors [5,8,10]. In general, there is a lack of chlamydia studies in young adolescent boys as most research has focused on girls [5].

In 2009, Norway had the third highest chlamydia notification rate in Europe (474/100 000) [1]. A chlamydia incidence rate almost twice the national average has been reported in Finnmark, the northernmost and most sparsely populated county in Norway [11]. The population includes ethnic Norwegians, indigenous Sami people, and minority groups of Kvens, Finns, and Russians living together in small municipalities. The highest annual incidence rates in Finnmark among females were observed in the age group 15–19 years, while among males the infections peaked in the 20–24 year age group [12]. This age and gender distribution is similar to surveillance data from most other Western European countries [1].

In this study, we hypothesized that a significant reservoir of undetected infections might exist among the sexually active adolescents in Finnmark, and that they might exhibit high levels of sexual risk behaviours to explain why chlamydia has remained endemic in the area. Our aims were to detect chlamydia prevalence in adolescent girls and boys in a high-incidence rural area, and second, to examine gender-specific early sexual behaviours associated with chlamydia infections that might contribute to disproportionate infection rates in girls.

## Methods

### Study population

A population based cross-sectional study was conducted in five high schools in five towns in Finnmark county

during fall 2009, reaching all high school students in these municipalities. In 2007–09, 94% of the birth cohorts in Finnmark county were enrolled in public high school, with an annual drop-out rate of approximately 10% [13]. This cross-sectional study was linked to a study on genetic diversity and distribution of *C. trachomatis* genotypes in the adolescent population in Norway. All chlamydia specimens had thus previously been genotyped using high resolution multilocus sequence typing, MLST [14].

Written information about the study in Norwegian and Sami was handed out in class by the teachers two weeks prior to data collection. All students regardless of sexual experience were invited to participate. From September 21<sup>st</sup> to November 19<sup>th</sup> 2009, the same study doctor and nurse consecutively visited all 123 classes in the high schools. As shown in Figure 1, participation rate was 98% (1,618 of 1,664) among the eligible students, while overall participation rate was 85% (1,618 of 1,908). A urine sample was provided by 93% of those reporting ever having had sexual intercourse with no gender difference. Among 6 students with inconclusive test results, one girl testing negative one day prior to the study was assumed to have a negative result and was included in the analysis. 5 boys with an inconclusive test did not provide a new urine sample and were excluded. 1,031 sexually active students aged 15–20 years with a valid chlamydia test result were included in the final study sample. Mean age was 17.2 years (median 17.0, SD 1.0).

### Questionnaire

On the day of data collection, a questionnaire designed in QuestBack online survey system ([www.questback.com](http://www.questback.com)) was emailed class-wise to the students. All Norwegian high school students have laptop computers with internet access making implementation feasible. Under supervision of the study staff and a teacher, participants spent 10–20 min completing the questionnaire which included questions on demography, sexual behaviour, alcohol and drug habits, prior chlamydia testing and treatment, and contraceptive use. Pre-programmed commands ensured automatic skipping of non-applicable questions. No reminders were sent.

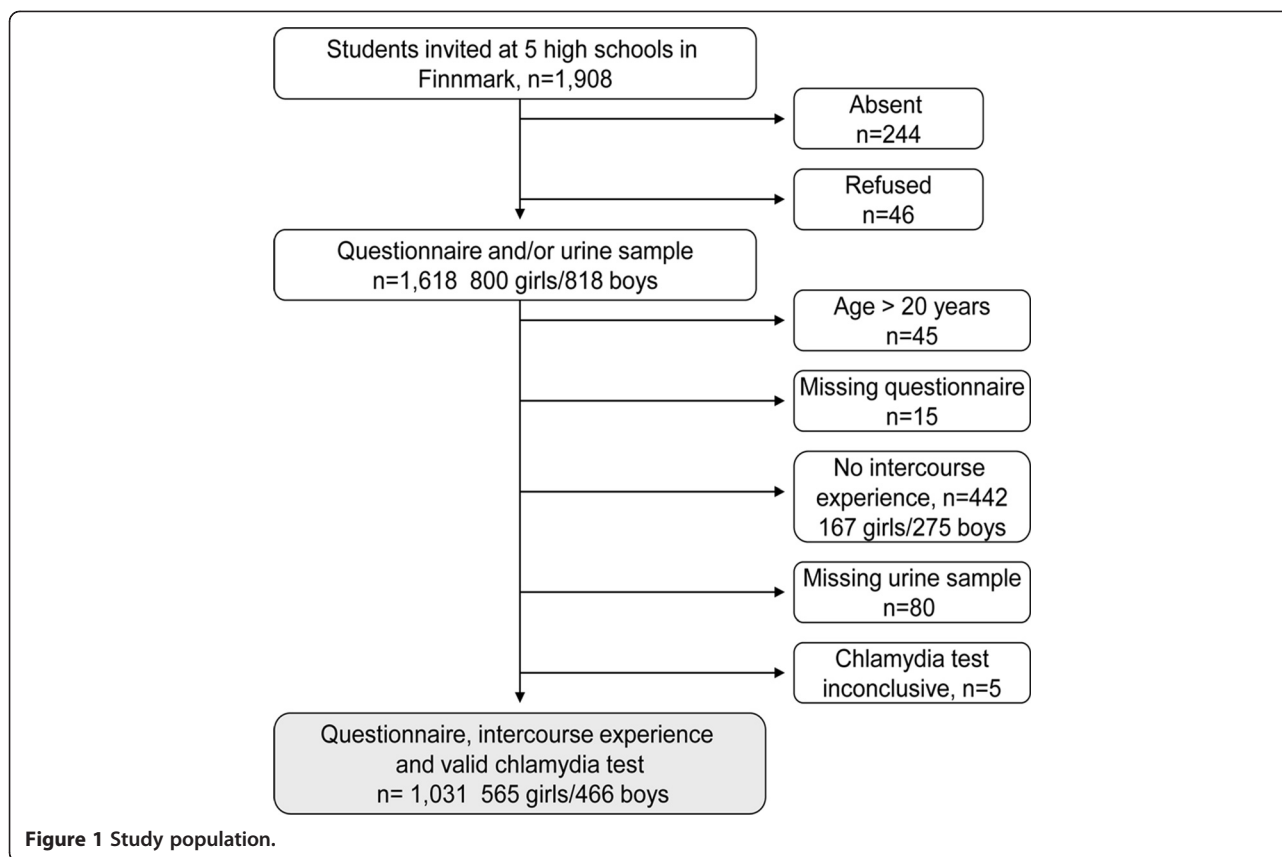
### Urine sampling

Directly thereafter, the participants went to the school toilets and provided first-void urine (FVU) samples under supervision of the nurse. The samples were immediately refrigerated and transported by National Mail Delivery on the same day to the University Hospital of North Norway, Tromsø, and analysed within 24 h.

### Chlamydia PCR

The laboratory extracted DNA using the BUGS'n BEADS TM-STI kit (NorDiag ASA, Oslo, Norway) and





**Figure 1** Study population.

used the ProCt real-time PCR (ProCelo as, Tromsø, Norway) with a sensitivity of 97% and a specificity of 100%.

#### Follow up

Participants with a positive chlamydia test result were given an appointment at the local youth clinic. A single dose of one gram azithromycin orally was either prescribed or handed out directly.

#### Sample size calculations

We estimated a sample size of 974 to achieve 90% power to detect a difference between an anticipated chlamydia prevalence of 3.0% in the source population irrespective of sexual intercourse experience, compared to 1.4% as observed in a similar study in South Norway using a 5% significance level [6]. The anticipated prevalence was based on a pilot study in Finnmark (unpublished data).

#### Data analysis

Descriptive characteristics were reported with means (SD) for continuous variables and with numbers (%) for categorical variables. The 95% confidence intervals (CI) for proportions were calculated using the exact binomial method. Crude and multivariable logistic regression models were applied with chlamydia test result as dependent

variable. Variables with  $p$  value  $<0.25$  in crude analysis were included in the multivariable models which were fitted using backward stepwise elimination. Age and gender (if applicable) were included regardless of significance. Collinearity was not a problem with variance inflation factor (VIF)  $<2.5$  for all variables. Gender interaction was assessed by including cross-product terms between each independent variable and gender. All statistical tests were two-sided using a 5% significance level and were performed in SPSS 19.0 (IBM Corp., New York, US).

Self-perceived ethnicity was coded in three categories based on the statement: 'I perceive my ethnicity as: Norwegian, Sami, Russian, Kven, Finnish, or other'. More than one answer was allowed. Category 'Norwegian' included those reporting Norwegian ( $n = 726$ ) and/or Kven ( $n = 5$ ) ethnicity, as the two share a common distribution of life-style factors [15]. 'Sami/Sami-Norwegian' included those reporting Sami ethnicity ( $n = 90$ ) or both Sami and Norwegian ethnicity ( $n = 139$ ). 'Other' included Russian ( $n = 19$ ), Finn ( $n = 20$ ) and other ( $n = 31$ ) ethnicity.

Use of alcohol, cannabis, amphetamine or ecstasy was reported for each substance as: never tried (1), tried (2), occasional use (3), or regular use (4). A new variable 'alcohol/drug use' was calculated as sum of the four substance use variables. Participants with missing response for alcohol ( $n = 5$ ) were excluded, but missing was

accepted for the other three. Range of the 'alcohol/drug use' variable was 2–16, and was defined as:  $\leq 5$ : 'low'; 6: 'medium';  $\geq 7$ : 'high'.

Condom use at first intercourse with first partner and at last intercourse with last partner were coded in two categories (yes/no) based on the question: 'Did you use any kind of contraception at first (last) sexual intercourse?' with response alternatives: 1) No; 2) Condom; 3) Hormonal contraception; 4) IUD; 5) Both condom and other contraception; 6) Emergency pill; 7) Coitus interruptus; 8) Don't know. Category 'yes' included participants with responses 2 and 5. 'No' included the remaining responses. 'Don't know' was answered by 3 girls and 10 boys at first intercourse and by 3 girls and 8 boys at last intercourse.

### Ethics

Written informed parental consent was obtained for participants <16 years. Participants  $\geq 16$  years gave their informed consent by filling in the web-based questionnaire. The study was approved by the Regional Committee for Medical and Health Research Ethics North Norway.

## Results

### Study sample

Socio-demographic characteristics of the study population are given in Table 1. Only participants at risk, i.e. reporting ever having had sexual intercourse, were included. Most participants reported Norwegian ethnicity (71%), while Sami/Sami-Norwegian was reported by 22%. More girls than boys chose an academic discipline rather than vocational (61% vs 37%,  $p < 0.001$ ). Boys more frequently reported high level of substance use (28% vs 19%,  $p < 0.001$ ). Sexual debut at  $\leq 14$  years was reported by 41% of girls and 34% of boys ( $p = 0.03$ ) (Table 2). More girls than boys had been sexually active for  $\geq 2$  years (73% vs 65%,  $p = 0.003$ ). 52% of girls and 36% of boys were currently in a steady relationship ( $p < 0.001$ ). More girls than boys reported  $\geq 6$  lifetime sexual partners (34% vs 25%,  $p = 0.003$ ). There was no gender difference in condom use at first intercourse, but more boys than girls reported condom use at last intercourse (34% vs 16%,  $p < 0.001$ ). Condom use at first sexual intercourse significantly increased the odds of condom use at last intercourse with last partner in both girls and boys (odds ratio, OR, 3.4 vs odds ratio, OR, 7.5,  $p = 0.002$ ). Last sexual partner  $\geq 1$  year older was reported by 76% of girls and 12% of boys ( $p < 0.001$ ). Average age of last partner was 19.8 years in girls and 16.4 years in boys ( $p < 0.001$ ). One-fourth of participants met last sex partner at a private party, bar or disco (girls 23%, boys 25%,  $p = 0.34$ ). More boys than girls had used alcohol or illicit drugs in connection with last intercourse (24% vs

18%,  $p = 0.03$ ). Girls were more likely than boys to have had a chlamydia test prior to the study (56% vs 21%,  $p < 0.001$ ), and to have received chlamydia treatment (20% vs 7%,  $p < 0.001$ ).

### Prevalence

*C. trachomatis* prevalence was 5.7%, in girls 7.3%, and in boys 3.9% (Table 1). This gives a prevalence of 4.1% (95% CI 3.3–5.3) in all participants irrespective of sexual intercourse experience. There were no statistically significant differences between the 5 schools. All participants with a positive test result reported sexual intercourse experience.

### Crude analyses

The following factors significantly increased the odds of infection in girls: Sami-Sami-Norwegian ethnicity, mothers education  $\geq$  college,  $\geq 2$  sexual partners past 6 months,  $\geq 6$  lifetime sexual partners, and meeting last partner at a private party, bar or disco (Tables 1 and 2). In boys, no condom use at first intercourse, no condom use with the most recent partner, and last sexual partner  $\geq 1$  year older increased the odds of chlamydia. Interaction was observed between gender and condom use at first intercourse ( $p = 0.003$ ), and was borderline significant for gender and maternal educational  $\geq$  college ( $p = 0.094$ ).

Assessing all participants, the following additional factors increased the odds of infection in crude analyses: female gender (OR 1.93, 95% CI 1.09–3.41), residence outside the family home in school year, and medium or high use of alcohol and illicit drugs.

### Multivariable logistic regression analysis

Among girls, mother's education  $\geq$  college,  $\geq 2$  sexual partners past 6 months, and meeting last sexual partner at a private party, bar or disco increased the likelihood of infection (Table 3). In boys, condom use at first intercourse with first partner decreased the odds of chlamydia while last sexual partner  $\geq 1$  year older increased the odds. In all participants, to have residence outside the family home,  $\geq 2$  sexual partners past 6 months, meeting last sexual partner at a private party, bar or disco, and condom use at last intercourse were significant.

### Discussion

We detected a substantial burden of chlamydia infections with a twofold higher prevalence in girls than in boys and with the infections beginning to be acquired soon after sexual initiation. The girls started to have sexual intercourse at younger age, had older partners, more frequently were in steady relationships, and reported higher numbers of lifetime partners than the boys. The

**Table 1 Sosio-demographic characteristics, prevalence and crude odds ratios for *C. trachomatis* infection in univariable logistic regression analysis**

Characteristic	Girls						Boys						Both				
	N	(%)	n <sub>CT</sub>	(%)	OR	95% CI	p <sup>1</sup>	N	(%)	n <sub>CT</sub>	(%)	OR	95% CI	p <sup>1</sup>	OR	95% CI	p <sup>1</sup>
<b>Total</b>	565	(100.0)	41	(7.3)				466	(100.0)	18	(3.9)						
<b>Age</b>																	
16	168	(29.7)	11	(6.5)	1.00		0.48	139	(29.8)	3	(2.2)	1.00		0.38	1.00		0.21
17	177	(31.3)	15	(8.5)	1.32	0.59 to 2.97		179	(38.4)	9	(5.0)	2.40	0.64 to 9.04		1.51	0.76 to 3.00	
18	166	(29.4)	9	(5.4)	0.82	0.33 to 2.03		106	(22.7)	3	(2.8)	1.32	0.26 to 6.68		0.97	0.44 to 2.13	
19-20	48	(9.6)	6	(11.1)	1.78	0.63 to 5.08		39	(9.0)	3	(7.1)	3.49	0.68 to 17.97		2.17	0.91 to 5.17	
<b>Family and culture</b>																	
<b>Ethnicity</b>																	
Norwegian	402	(71.2)	23	(5.7)	1.00		0.093	325	(70.0)	12	(3.7)	1.00		0.73	1.00		0.21
Sami/Sami-Norwegian	124	(21.9)	14	(11.3)	2.10	1.04 to 4.21		105	(22.6)	3	(2.9)	0.74	0.21 to 2.77		1.59	0.87 to 2.89	
Other	39	(6.9)	4	(10.3)	1.88	0.61 to 5.75		34	(7.3)	2	(5.9)	1.63	0.35 to 7.61		1.77	0.72 to 4.36	
<b>Church affiliation<sup>2</sup></b>																	
Yes	448	(79.6)	33	(7.4)	1.00		0.88	294	(63.5)	12	(4.1)	1.00		0.54	1.00		0.36
No	115	(20.4)	8	(7.0)	0.94	0.42 to 2.10		169	(36.5)	5	(3.0)	0.72	0.25 to 2.07		0.74	0.40 to 1.40	
<b>Residence in school year</b>																	
At home	356	(63.1)	20	(5.6)	1.00		0.051	287	(61.7)	7	(2.4)	1.00		0.084	1.00		0.011
Other <sup>3</sup>	208	(36.9)	21	(10.1)	1.89	1.00 to 3.57		178	(38.3)	10	(5.6)	2.38	0.89 to 6.37		2.00	1.17 to 3.4	
<b>Mother's education</b>																	
≤ High school/don't know	313	(55.5)	15	(4.8)	1.00		0.013	304	(65.7)	12	(3.9)	1.00		0.66	1.00		0.03
≥ College	251	(44.5)	26	(10.4)	2.30	1.19 to 4.44		159	(34.3)	5	(3.1)	0.79	0.27 to 2.28		1.79	1.05 to 3.04	
<b>High school</b>																	
<b>Study category</b>																	
Academic	347	(61.4)	21	(6.1)	1.00		0.17	171	(36.8)	6	(3.5)	1.00		0.90	1.00		0.56
Vocational	218	(38.6)	20	(9.2)	1.57	0.83 to 2.97		294	(63.2)	11	(3.7)	1.07	0.39 to 2.94		1.17	0.69 to 1.99	
<b>Year</b>																	
1	210	(37.2)	16	(7.6)	1.00		0.68	178	(38.3)	4	(2.2)	1.00		0.44	1.00		0.83
2	198	(35.0)	16	(8.1)	1.07	0.52 to 2.19		209	(44.9)	9	(4.3)	1.96	0.59 to 6.47		1.20	0.66 to 2.21	
3	157	(27.8)	9	(5.7)	0.74	0.32 to 1.72		78	(16.8)	4	(5.1)	2.35	0.57 to 9.65		1.08	0.53 to 2.21	
<b>Alcohol/drug use</b>																	
Low	134	(23.8)	4	(3.0)	1.00		0.098	130	(28.4)	3	(2.3)	1.00		0.087	1.00		0.034
Medium	322	(57.3)	27	(8.4)	2.98	1.02 to 8.67		198	(43.3)	5	(2.5)	1.10	0.26 to 4.67		2.41	1.05 to 5.53	
High	106	(18.9)	10	(9.4)	3.39	1.03 to 11.12		129	(28.2)	9	(7.0)	3.18	0.84 to 12.01		3.23	1.33 to 7.83	

n<sub>CT</sub> number of chlamydia cases, OR odds ratio, CI confidence interval, <sup>1</sup>p-value for equality between categories; <sup>2</sup>Affiliation to any denomination: Church of Norway (n = 722), Russian Orthodox Church (n = 9), Jehova's Witnesses/Pentecostals (n = 7), Islam (n = 4); <sup>3</sup>Living with relatives, in students' houses or in private accommodation.

**Table 2 Sexual behaviour, prevalence and crude odds ratios for *C. trachomatis* infection in univariable logistic regression analysis**

Characteristic	Girls							Boys							Both		
	N	(%)	n <sub>CT</sub>	(%)	OR	95% CI	<i>p</i> <sup>1</sup>	N	(%)	n <sub>CT</sub>	(%)	OR	95% CI	<i>p</i> <sup>1</sup>	OR	95% CI	<i>p</i> <sup>1</sup>
<b>Sexual behaviour</b>																	
Age at first intercourse																	
≥ 15 years	330	(58.8)	24	(7.3)	1.00		0.97	294	(65.9)	8	(2.7)	1.00		0.10	1.00		0.27
≤ 14 years	231	(41.2)	17	(7.4)	1.01	0.53 to 1.93		152	(34.1)	9	(5.9)	2.25	0.85 to 5.96		1.35	0.79 to 2.30	
Years sexually active																	
≤ 1 year	149	(26.6)	10	(6.7)	1.00		0.74	158	(35.4)	2	(1.3)	1.00		0.055	1.00		0.099
≥ 2 years	412	(73.4)	31	(7.5)	1.13	0.54 to 2.37		288	(64.6)	15	(5.2)	4.29	0.97 to 18.99		1.73	0.90 to 3.31	
Condom use first intercourse																	
No <sup>2</sup>	223	(39.6)	14	(6.3)	1.00		0.46	206	(45.3)	16	(7.8)	1.00		0.003	1.00		0.13
Yes	340	(60.4)	27	(7.9)	1.29	0.66 to 2.51		249	(54.7)	1	(0.4)	0.05	0.01 to 0.36		0.75	0.39 to 1.13	
Currently in a relationship																	
Yes	296	(52.4)	19	(6.4)	1.00		0.42	167	(35.8)	6	(3.6)	1.00		0.82	1.00		0.69
No	269	(47.6)	22	(8.2)	1.30	0.69 to 2.46		299	(64.2)	12	(4.0)	1.12	0.41 to 3.05		1.12	0.66 to 1.90	
Sex partners past 6 months																	
0-1	323	(57.9)	12	(3.7)	1.00		<0.001	254	(58.8)	6	(2.4)	1.00		0.053	1.00		<0.001
≥ 2	235	(42.1)	29	(12.3)	3.65	1.82 to 7.31		178	(41.2)	11	(6.2)	2.72	0.99 to 7.50		3.33	1.88 to 5.90	
Lifetime no of sex partners																	
1-2	192	(34.5)	6	(3.1)	1.00		0.002	203	(47.8)	4	(2.0)	1.00		0.24	1.00		<0.001
3-5	178	(32.0)	11	(6.2)	2.04	0.74 to 5.64		117	(27.5)	4	(3.4)	1.76	0.43 to 7.18		2.06	0.91 to 4.66	
≥ 6	186	(33.5)	24	(12.9)	4.59	1.83 to 11.51		105	(24.7)	6	(5.7)	3.02	0.83 to 10.93		4.43	2.13 to 9.21	
<b>Last sexual partner</b>																	
Age difference																	
Same age or younger	133	(24.3)	7	(5.3)	1.00		0.30	381	(88.0)	11	(2.9)	1.00		0.005	1.00		0.002
Older (≥ 1 year)	414	(75.7)	33	(8.0)	1.56	0.67 to 3.61		52	(12.0)	6	(11.5)	4.39	1.55 to 12.42		2.52	1.42 to 4.47	
How met last partner																	
At school/work	66	(11.7)	2	(3.0)	1.00		0.008	104	(23.0)	2	(1.9)	1.00		0.65	1.00		0.008
Through family/friends/other	312	(55.4)	16	(5.1)	1.73	0.39 to 7.71		187	(41.4)	4	(4.2)	1.98	0.40 to 9.73		2.01	0.68 to 5.89	
On the Internet	58	(10.3)	5	(8.6)	3.02	0.56 to 16.19		47	(10.4)	2	(4.3)	2.27	0.31 to 16.60		2.96	0.85 to 10.38	
At a private party, bar, disco	127	(22.6)	18	(14.2)	5.28	1.19 to 23.52		114	(25.2)	6	(5.3)	2.83	0.56 to 14.36		4.59	1.56 to 13.48	
Type of relationship																	
Regular partner/sweetheart	324	(57.4)	20	(6.2)	1.00		0.31	209	(46.3)	5	(2.4)	1.00		0.090	1.00		0.51
Ex partner/sweetheart	74	(13.1)	8	(10.8)	1.84	0.78 to 4.36		51	(11.3)	1	(2.0)	0.82	0.093 to 7.14		1.58	0.72 to 3.47	
A friend I have sex with	103	(18.3)	6	(5.8)	0.94	0.37 to 2.41		95	(21.1)	8	(8.4)	3.75	1.19 to 11.79		1.55	0.79 to 3.04	
Casual contact/other	63	(11.2)	7	(11.1)	1.91	0.77 to 4.71		96	(21.3)	3	(3.1)	1.32	0.31 to 5.62		1.36	0.64 to 2.90	

**Table 2 Sexual behaviour, prevalence and crude odds ratios for *C. trachomatis* infection in univariable logistic regression analysis (Continued)**

<b>Last sexual intercourse</b>																
Condom use																
No <sup>3</sup>	474	(84.0)	39	(8.2)	1.00		0.062	300	(66.5)	16	(5.3)	1.00		0.039	1.00	0.003
Yes	90	(16.0)	2	(2.2)	0.25	0.06 to 1.07		151	(33.5)	1	(0.7)	0.12	0.02 to 0.90		0.17	0.05 to 0.53
Related alcohol/drug use																
No	460	(81.7)	30	(6.5)	1.00		0.15	343	(76.2)	11	(3.2)	1.00		0.26	1.00	0.10
Yes	103	(18.3)	11	(10.7)	1.71	0.83 to 3.54		107	(23.8)	6	(5.6)	1.79	0.65 to 4.97		1.64	0.91 to 2.94
<b>Chlamydia infection</b>																
Test prior to study																
No	251	(45.5)	18	(7.2)	1.00		0.94	368	(79.1)	11	(3.0)	1.00		0.14	1.00	0.11
Yes	313	(55.5)	23	(7.3)	1.03	0.54 to 1.95		97	(20.9)	6	(6.2)	2.14	0.77 to 5.94		1.55	0.91 to 2.63
Treatment for infection																
No	452	(80.1)	30	(6.6)	1.00		0.25	433	(93.3)	16	(3.7)	1.00		0.87	1.00	0.13
Yes	112	(19.9)	11	(9.8)	1.53	0.74 to 3.16		31	(6.7)	1	(3.2)	0.87	0.11 to 6.78		1.67	0.86 to 3.24

n<sub>CT</sub>, number of chlamydia cases, OR, odds ratio, CI, confidence interval, <sup>1</sup>p-value for equality between categories, <sup>2</sup>Includes the response: 'Uncertain if any contraception was used' (girls n = 3, boys n = 10), <sup>3</sup>Includes the response: 'Uncertain if any contraception was used' (girls n = 3, boys n = 8).

**Table 3 Odds ratios for *C. trachomatis* infection in multivariable logistic regression models**

Characteristic	Girls			Boys			Both		
	OR	95% CI	<i>p</i> <sup>1</sup>	OR	95% CI	<i>p</i> <sup>1</sup>	OR	95% CI	<i>p</i> <sup>1</sup>
<b>Family and culture</b>									
Residence in school year									
At home	-			-			1.00		0.013
Other <sup>2</sup>	-			-			2.04	1.17–3.57	
Mother's education									
≤High school/don't know	1.00		0.021	-			-		
≥College	2.22	1.13–4.37		-			-		
<b>Sexual behaviour</b>									
Condom use first intercourse									
No	-			1.00		0.005	-		
Yes	-			0.06	0.01–0.42		-		
Sex partners past 6 months									
0-1	1.00		<0.001	-			1.00		<0.001
≥ 2	3.59	1.76–7.32		-			2.88	1.60–5.18	
<b>Last sexual partner</b>									
Age difference									
Same age or younger	-			1.00		0.017	-		
Older (≥1 year)	-			3.74	1.27–11.01		-		
How met last partner									
At school/work	1.00		0.038	-			1.00		0.026
Through family/friends/other	1.90	0.42–8.65		-			1.61	0.54–4.82	
On the Internet	3.40	0.62–18.78		-			2.81	0.78–10.08	
At a private party, bar, disco	4.99	1.10–22.69		-			3.54	1.18–10.61	
<b>Last sexual intercourse</b>									
Condom use									
No	-			-			1.00		0.015
Yes	-			-			0.23	0.07–0.75	

OR odds ratio, CI confidence interval, <sup>1</sup>*p*-value for equality between categories; <sup>2</sup>Living with relatives, in students' houses or private accommodation. The multivariable models include the significant variables from backwards stepwise procedure. Age and gender (if applicable) included in all models.

boys claimed more substance use related to last intercourse and overall, had same-aged or younger partners, and remained better condom users. Accordingly, girls and boys had differing independent risk factors for chlamydia infection.

### Prevalence

A chlamydia prevalence of 5.7% was significantly higher than detected in two high school studies in South Norway; 2.0%, and Luxembourg; 1.9%, and in a population based Dutch study in age group 15–19 years; 2.9% [6,7,9]. It is more comparable to 5.2% detected in a high school study in urban Philadelphia, USA [8]. The high prevalence is in line with the high incidence rates observed in surveillance data, and was to be expected as adolescents living in high prevalence STI areas have significantly increased odds of having a current STI given the available pool of infected partners [16]. A twofold

higher prevalence in girls is similar to the results in the above mentioned studies [6-9]. However, 7.3% is probably a minimum estimate in the female participants as *C. trachomatis* was detected in FVU samples that are 10% less sensitive than self-collected vaginal swabs [17].

### Socio-demographic characteristics

Sami/Sami-Norwegian girls having twice the prevalence of ethnic Norwegian girls is in line with a surveillance-based study from 1993 that observed a 6 times higher chlamydia incidence in a Sami municipality in Finnmark compared to the national average [18]. The Sami/Sami-Norwegian girls more frequently lived outside the family home and reported higher numbers of lifetime sexual partners than the Norwegian girls.

One-third of all participants lived in villages without high schools and had left home to attain further education, and these participants had twice the odds of infection

compared to those living at home. To our knowledge, this has not been assessed in previous studies. Lack of parental control and detachment from the norms of their community of origin may explain the observed differences.

Maternal educational level  $\geq$  college was associated with a twofold higher prevalence in girls, but not in boys. Daughters of higher educated mothers reported more substance use overall and in connection with last intercourse than those with less educated mothers. In contrast, maternal education  $\geq$  college level was shown to protect against STIs in a longitudinal study in the USA [19]. The opposing results may reflect cultural differences regarding sexual norms with higher educated mothers in the Nordic countries leaving their daughters more freedom than their American counterparts.

### Sexual behaviour

Condom use at first intercourse was a significantly better predictor of condom use at last intercourse in boys than in girls and can partly explain why condom use at sexual debut was highly protective against chlamydia only in the boys' multivariable model. The poorer predictability of condom use at last intercourse in girls and the finding that more boys than girls used condoms at last intercourse may indicate that girls switch to hormonal contraceptives. Adolescent girls may also lack power to negotiate safe sex with their mostly older partners [5]. Condom use at last intercourse with last partner may be associated with use at previous sexual encounters and thereby explain the protective effect against chlamydia observed in all participants. Most studies show that condom use is associated with reduced chlamydia risk in both women and men [20].

As observed in other studies, number of sexual partners past 6 months was strongly associated with chlamydia infection in girls [7,21]. The lack of association in boys could be due to boys frequently over-reporting their number of sexual partners [22].

A higher number of gender-specific *C. trachomatis* genotypes had previously been detected in girls than in boys in this study population [14]. Based on the genotyping results and most girls reporting older last sexual partners, we concluded that the girls were linked to off-school sexual networks with a different genotype reservoir than same-age boys. As chlamydia infections in surveillance data peak in males aged 20–24 years, we assumed that the older male partners would have higher chlamydia rates than our high school boys. Accordingly, we expected that having an older partner would increase the odds of infection in girls [12]. Due to less than one-fourth of girls having last sexual partner same age or younger, the increased infection risk in adolescent girls usually associated with age disparities may have been

obscured [23]. To our knowledge, this is the first study to apply high-resolution genotyping as biological support for participants' self-reported sexual behaviour in a population based study. Only 12% of the boys reported last sexual partner  $\geq 1$  year older, but this increased the odds of chlamydia threefold in boys and is similar to the results observed in a recent study [24]. The increase in odds disappeared when adjusting for number of partners past 6 months, indicating that adolescent boys who attract older women may have more opportunities for sex and hence are more sexually active than peers with younger or same-aged partners.

An increased infection risk associated with sexual partners met at a private party, bar or disco could reflect high-risk sexual behaviours and higher chlamydia prevalence among individuals who frequent these venues [23].

Young age at first sexual intercourse is a commonly reported risk factor for chlamydia in adolescents [25]. The Nordic countries traditionally have a higher acceptance of both female and adolescent sexuality than most other Western industrialized countries and are often regarded as representing liberated cultures [4,26]. More than 40% of the sexually active girls in our study reporting sexual debut at  $\leq 14$  years may indicate that sexual activity in adolescent girls is accepted in these communities and could explain why early sexual debut did not appear as a risk factor in girls. This is supported by a recent study showing that early coital debut was independently associated with living in Northern Norway [27]. In the boys' crude analysis, early first intercourse was only borderline significant.

The following factors were assumed to be important for the unusually high participation rate in our study: the school-based setting, a test result notification time of only 1–2 days, and class-wise data collection by the same professional study staff.

This is one of few population based studies on prevalent chlamydia infections and associated sexual behaviours in Europe covering both girls and boys aged 15–20 years. We showed that girls and boys accumulate different experiences early in their sexual careers which contribute to the differing chlamydia risk. It confirmed traditional factors commonly associated with chlamydia (female gender, multiple sex partners, older partners, no condom use), but also detected less studied demographic characteristics (residence outside the family home, maternal education) and risk factors (meeting venues for sexual partners).

### Limitations

The study is limited by the cross-sectional design that precludes establishing causality, the self-reported behavioural data, and the lack of statistical power with only 41 chlamydia cases in girls and 18 in boys. Although



the use of a web-based questionnaire is likely to have reduced social desirability bias [28], sensitive information on sexual behaviour and substance use were self-reported and could be prone to such bias. Finally, our findings may be applicable mainly to the Nordic countries as sexual behaviour has been shown to vary between different cultures and countries [29].

### Conclusions and recommendations

In conclusion, girls this age may be the most cost-effective targets for preventive measures and screening due to a high burden of infections and our finding that young girls often make poor choices regarding their sexual health. However, young boys should also be targeted to make them partners in STI control early on. Gender-specific approaches to control chlamydia infections at this particular age may be the best alternative.

### Abbreviations

FVU: First-void urine; STI: Sexually transmitted infection.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

KG conceived and designed the study, collected the data, and drafted the manuscript. GSS and ASF participated in study design. KG and TW performed the statistical analyses. All authors contributed to the interpretation of the results, and revised and approved the manuscript.

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# Paper III



RESEARCH ARTICLE

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# Factors associated with *Chlamydia trachomatis* testing in a high school based screening and previously in clinical practice: a cross-sectional study in Norway

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## Abstract

**Background:** High school based chlamydia screening has been shown to increase uptake and detect hidden infections among sexually active adolescents. Our study aimed to: i) examine the proportions of 15–20 year-olds tested in a high school based screening and previously in clinical practice, ii) determine chlamydia prevalence according to testing pattern, and iii) examine factors associated with testing in the two settings.

**Methods:** A population based cross-sectional study was conducted in 5 high schools in Norway in 2009, using web-questionnaires and *Chlamydia trachomatis* PCR in first-void urine (800 girls/818 boys, mean age 17.2 years). Only sexually active participants at risk for chlamydia infections were included in the analyses. Crude and multivariable logistic regression models were applied with 'clinic based testing' and 'school based screening' as outcome variables.

**Results:** 56% of girls and 21% of boys reported previous clinic based testing. In the school based screening, 93% were tested with no gender difference. 42% of girls and 74% of boys were tested for the first time at school ('school-only test'). Both girls with clinic based testing and girls with school-only test had high chlamydia prevalence (7.3% vs 7.2%). Boys with clinic based testing had twice the prevalence of those with school-only test (6.2% vs 3.0%,  $p = 0.01$ ). Half of infections were detected in participants with school-only test. One-fifth were repeat infections. In multivariable analysis of girls and boys combined, female gender, older age, early sexual debut, no condom use at first and last intercourse, steady relationship, and higher number of lifetime partners increased the odds of clinic based testing. The odds of school based screening increased with male gender, academic affiliation, later sexual debut, condom use at first intercourse, and current urogenital symptoms in multivariable analysis.

**Conclusions:** More than half the girls had been tested prior to the school based screening and had high prevalence independent of previous clinic based testing. School screening was mostly associated with factors unknown to increase chlamydia infection risk, while clinic based testing was associated with traditional risk factors. The unusually high and equal participation between genders and the detection of a large chlamydia reservoir confirms the value of school based screening suggesting this approach to be further explored in Norway.

**Keywords:** Chlamydia trachomatis, Testing, High school screening, Adolescents, Gender differences

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## Background

Adolescents have a disproportionate burden of genital *Chlamydia trachomatis* infections, but may not feel at risk since most infections are asymptomatic and testing rates remain low [1-3]. Repeat chlamydia infections are common in adolescents [4]. According to Norwegian surveillance data, the female to male chlamydia test ratio has been more than 4 to 1 in age group 15–19 years with average positivity rates of 15% in girls and 18% in boys, respectively [2]. Accurate annual testing rates and rates of repeat testing in the Norwegian adolescent population cannot be estimated due to lack of unique personal identifiers. The major predominance of females is in line with chlamydia screening programmes in other high-income countries and may reflect gender differences in health seeking behaviour [5,6].

Norwegian health authorities recommend chlamydia testing in the presence of clinical symptoms, or if partner is infected, or in persons younger than 25 years if change in sexual partner [7]. Testing and treatment in these groups are free of charge. Test of cure is recommended 5–6 weeks after treatment. The majority of chlamydia testing among adolescents is done in general practice and in public youth clinics which are tailored to the needs of adolescents and are present in most municipalities. Youth clinics offer contraceptive counselling without parental consent and all services are free. Most high schools have a school nurse available part time providing general health services that only include limited chlamydia testing. Sexually transmitted infection (STI) clinics are available only in a few large towns.

Expansion of chlamydia testing from clinical practices to school based settings has been shown to increase uptake among adolescents, particularly in boys [8-12]. A number of extensive chlamydia screening programmes have been implemented in high schools in the US [9-11], but less so in Europe [8,13]. In 2009, we conducted a cross-sectional study on early sexual behaviour and chlamydia infection among high school students in Norway. Among the sexually active, chlamydia prevalence was 7.3% (95% confidence interval, CI, 5.3–9.7%) in girls and 3.9% (2.3–6.0) in boys with infections starting to be acquired soon after sexual initiation [14]. This study provided an opportunity to examine factors associated with chlamydia testing in a school based screening and previously in clinical practice, and to estimate the chlamydia reservoir in adolescents not seeking testing on their own. As school based chlamydia screening is not current policy in Norway, we assumed that previous testing had been done in clinical practice, ie 'clinic based testing'.

The objectives of this paper were to; i) examine the proportion of adolescents aged 15–20 years in Norway tested in a high school based screening and previously in clinical practice, ii) determine chlamydia prevalence

according to testing pattern, and iii) examine demographic and sexual behavioural characteristics associated with school based screening and previous clinic based testing.

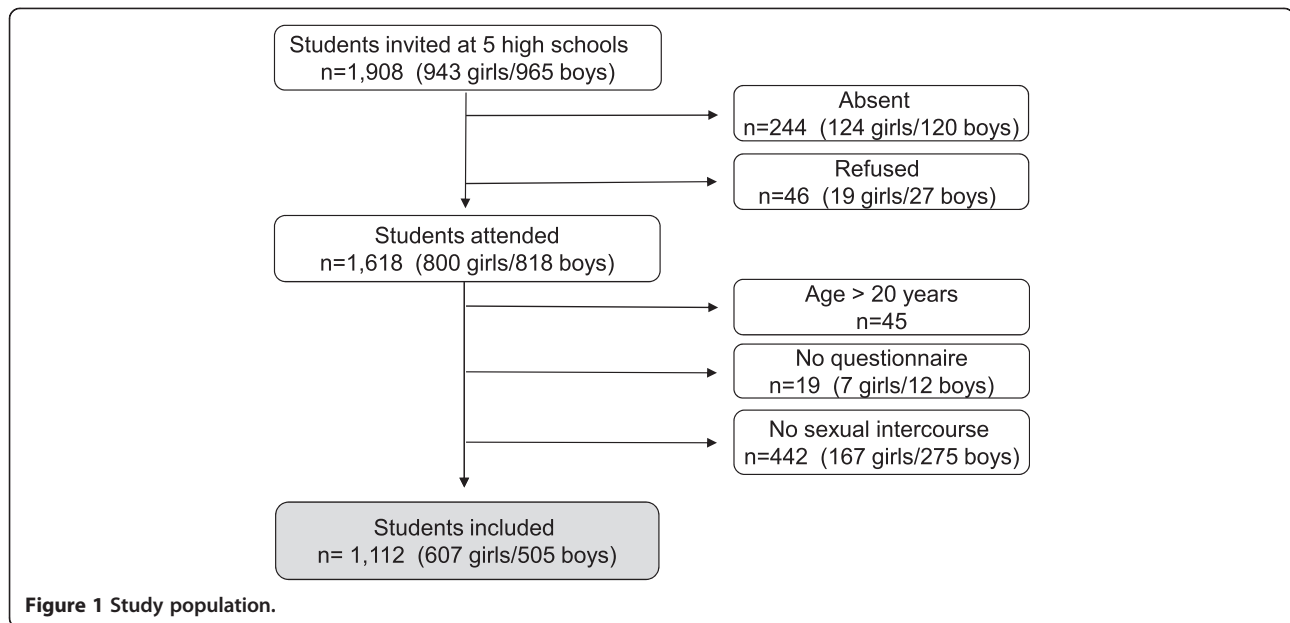
## Methods

A detailed description of the study has been reported elsewhere [14]. In brief, a population based cross-sectional study was conducted in 5 public high schools in Finnmark county in Northern Norway in 2009 using a web-questionnaire and first-void urine (FVU) samples. All data were collected by the same experienced female doctor and nurse who consecutively visited a total of 123 classes using an identical approach. Written information about chlamydia infection, questionnaire items, and sampling procedures were handed out in class two weeks prior to data collection. Confidentiality regarding questionnaire data and chlamydia test results was assured both in the written information and later by oral repetition in each class. On the day of data collection, a web-questionnaire was emailed class-wise to each student including questions on demography, substance use, sexual behaviour, contraceptive use, current urogenital symptoms, and prior chlamydia testing and treatment. The teacher and study staff were present in class while participants filled in the questionnaire on their laptops. Directly thereafter, participants went on to the school toilets where they provided about 12 ml FVU samples under supervision of the study nurse. Samples were immediately refrigerated and delivered to the laboratory on the following day for *C. trachomatis* PCR testing (ProCt real-time PCR, ProCelo as, Tromsø, Norway). Test result notification time was 1–2 days. Participants testing positive were called on their cell phone by the nurse and given an appointment at the local youth clinic. Infections were treated with a single dose of 1 gram azithromycin orally.

Overall participation rate was 85% (1,618 of 1,908) (Figure 1). If only assessing students present at school, 2% (46 of 1,664) refused participation. 442 participants responding 'no' to: 'Have you ever had sexual intercourse?' were considered not to be at risk for chlamydia infection and were excluded from the analyses. All 442 had negative test results. 1,112 participants reporting sexual intercourse experience were considered to be at risk and were included. Mean age was 17.2 years (standard deviation, SD 1.0, median age 17.0 years).

The variable 'high school study affiliation' was defined as; 1) 'academic', including students in the general academic studies programme, and 2) 'vocational', including vocational school students. In Norway, academic and vocational classes frequently share facilities throughout high school.

Previous clinic based testing was assessed by; 'Have you previously been tested for genital chlamydia infection?'



**Figure 1** Study population.

with response options: 'Yes, once', 'Yes, twice', 'Yes, 3 times', 'Yes  $\geq 4$  times', or 'No'. Due to small groups, the variable 'clinic based testing' was dichotomised as yes/no. 'School based screening' included all participants that were screened in the high school study independent of clinic based testing. The subgroup 'school-only test' included participants with no previous clinic based testing that provided a urine sample in the school based screening.

### Statistical analysis

Descriptive characteristics were reported with means (SD) for continuous variables and with numbers (%) for categorical variables. The 95% CI for proportions were calculated using the exact binominal method. Crude and multivariable logistic regression models were applied with two dependent variables: 1) 'clinic based testing'; yes/no, and 2) 'school based screening'; yes/no. All analyses were performed separately for girls and boys and in both genders combined. Variables with  $p$  value  $< 0.25$  in crude analysis were included in the multivariable models which were fitted using stepwise elimination. Age and gender (if applicable) were included regardless of significance. Collinearity was not a problem with variance inflation factor (VIF)  $< 2.5$  for all variables. Gender interaction was assessed by including cross-product terms between each independent variable and gender. Statistically significant interaction terms were included in the final multivariable model. Model fit was assessed using Hosmer and Lemeshow goodness-of-fit test with 5 of 6  $p$  values  $> 0.25$ . All statistical tests were two-sided using a 5% significance level and were performed in SPSS 19.0 (IBM Corp., New York, US).

### Ethics

Written informed parental consent was obtained for participants  $< 16$  years. Participants  $\geq 16$  years gave their informed consent by filling in the web-based questionnaire. The study was approved by the Regional Committees for Medical and Health Research Ethics North Norway.

### Results

Clinic based chlamydia testing was reported by 56% of girls and 21% of boys (Table 1) with more girls than boys reporting multiple tests (61% vs 34%,  $p < 0.001$ ). In the high school based screening, 93% of sexually active participants, 564 of 607 girls and 470 of 505 boys, were tested with no gender difference (Additional file 1). 42% of girls and 74% of boys were tested for the first time in the school based screening, i.e. school-only test.

### Chlamydia prevalence

Among participants with previous clinic based testing, chlamydia prevalence was 7.3% (95% CI 4.7–10.8) in girls and 6.2% (2.3–13.0) in boys. Among participants with school-only test, prevalence was 7.2% (4.3–11.1) in girls, and 3.0% (1.5–5.3) in boys. 50% ( $n = 29$ ) of the chlamydia infected participants reported clinic based testing and 21% ( $n = 12$ ) reported previous treatment. Among 41 girls with a positive chlamydia test result in the school based screening, 23 reported clinic based testing (Table 1). Among 17 boys screening positive at school, 6 reported previous clinic based testing.

### Clinic based testing

In gender-stratified crude analysis, the following variables increased the odds of clinic based testing in both

**Table 1 Sociodemographic and sexual behaviour characteristics - prevalence and crude odds ratios for clinic based testing**

Characteristic	Girls						Boys						All participants	
	Clinic based testing						Clinic based testing						p <sup>1</sup>	Interaction p <sup>2</sup>
	N	n	(%)	OR	95% CI	p <sup>1</sup>	N	n	(%)	OR	95% CI	p <sup>1</sup>		
<b>Total</b>	607	338	(55.7)	NA			505	106	(21.0)	NA				
<b>Age</b>														
15-16	179	81	(45.3)	1.00		< 0.001	149	19	(12.8)	1.00		0.001	< 0.001	0.80
17	193	105	(54.4)	1.44	0.96–2.17		196	40	(20.4)	1.75	0.97–3.18			
18	177	104	(58.8)	1.72	1.13–2.62		114	28	(24.6)	2.23	1.17–4.24			
19-20	58	48	(82.8)	5.81	2.77–12.20		46	19	(41.3)	4.81	2.25–10.29			
<b>Family and culture</b>														
Ethnicity														
Norwegian	433	232	(53.6)	1.00		0.049	353	56	(15.9)	1.00		< 0.001	0.001	0.022
Sami/Sami-Norwegian	131	85	(64.9)	1.60	1.07–2.40		115	37	(32.2)	2.52	1.55–4.08			
Other	43	21	(48.8)	0.83	0.44–1.55		36	13	(36.1)	3.00	1.43–6.27			
Residence in school year														
At home	380	196	(51.6)	1.00		0.007	314	55	(17.5)	1.00		0.015	0.001	0.78
Other <sup>3</sup>	226	142	(62.8)	1.59	1.13–2.22		191	51	(26.7)	1.72	1.11–2.65			
Mothers education														
≤ High school/don't know	338	184	(54.4)	1.00		0.46	334	62	(18.6)	1.00		0.074	0.005	0.32
≥ College	268	154	(57.5)	1.13	0.82–1.56		169	43	(25.4)	1.50	0.96–2.33			
<b>High school</b>														
Study affiliation														
Academic	323	189	(52.1)	1.00		0.029	181	45	(24.9)	1.00		0.11	0.040	0.010
Vocational	244	149	(61.1)	1.44	1.04–2.01		324	61	(18.8)	0.70	0.45–1.09			
<b>Alcohol/drug use</b>														
Low	144	57	(40.4)	1.00		< 0.001	140	21	(15.3)	1.00		0.003	< 0.001	0.49
Medium	343	193	(56.3)	1.90	1.27–2.82		212	40	(18.9)	1.29	0.72–2.29			
High	118	86	(72.9)	3.96	2.34–6.71		144	45	(31.3)	2.51	1.40–4.50			
<b>Sexual behaviour</b>														
Age at first intercourse														
≥ 15 years	352	167	(47.4)	1.00		< 0.001	311	40	(12.9)	1.00		< 0.001	< 0.001	0.15
≤ 14 years	252	171	(67.9)	2.34	1.67–3.28		172	59	(34.3)	3.54	2.24–5.59			
Years sexually active														
≤ 1 year	162	54	(33.3)	1.00		< 0.001	171	13	(7.6)	1.00		< 0.001	< 0.001	0.50
≥ 2 years	442	284	(64.3)	3.60	2.46–5.26		312	86	(27.6)	4.63	2.50–8.58			
Condom use first intercourse														
Yes	358	198	(55.3)	1.00		0.78	267	40	(15.0)	1.00		0.001	0.15	0.014
No <sup>4</sup>	248	140	(56.5)	1.05	0.76–1.45		226	61	(27.0)	2.10	1.34–3.28			
Currently in relationship														
Yes	322	185	(57.5)	1.00		0.35	179	42	(23.5)	1.00		0.31	0.001	0.79
No	285	153	(53.7)	0.86	0.62–1.18		326	64	(19.6)	0.80	0.51–1.24			



**Table 1 Sociodemographic and sexual behaviour characteristics - prevalence and crude odds ratios for clinic based testing (Continued)**

Sex partners past 6 months													
0-1	352	181	(51.4)	1.00		0.010	273	35	(12.8)	1.00	< 0.001	< 0.001	0.013
≥ 2	248	154	(62.1)	1.55	1.11–2.16		194	62	(32.0)	3.19	2.01–5.09		
Life time no of sex partners													
1-2	206	64	(31.1)	1.00		< 0.001	218	11	(5.0)	1.00	< 0.001	< 0.001	0.17
3-5	191	107	(56.0)	2.83	1.87–4.26		123	31	(25.2)	6.34	3.06–13.16		
≥ 6	201	163	(81.1)	9.52	6.01–15.08		119	53	(44.5)	15.11	7.46–30.62		
<b>Last sexual partner</b>													
Age difference													
Same age or younger	144	66	(45.8)	1.00		0.005	413	80	(19.4)	1.00	0.042	< 0.001	0.78
Older (≥ 1 year)	445	264	(59.3)	1.72	1.18–2.52		54	17	(31.5)	1.91	1.03–3.57		
Condom use last intercourse													
Yes	94	33	(35.1)	1.00		< 0.001	319	76	(23.8)	1.00	0.009	< 0.001	0.36
No <sup>5</sup>	513	305	(59.5)	2.71	1.71–4.29		168	23	(13.7)	1.97	1.18–3.28		
<b>School based testing</b>													
Provision of urine sample													
Yes	564	313	(55.5)	1.00		0.74	470	97	(20.6)	1.00	0.48	0.49	0.73
No	43	25	(58.1)	1.11	0.59–2.09		35	9	(25.7)	1.33	0.60–2.93		
Chlamydia test result													
Negative	523	290	(55.4)	1.00		0.94	448	91	(20.3)	1.00	0.14	0.11	0.23
Positive	41	23	(56.1)	1.03	0.54–1.95		17	6	(35.3)	2.14	0.77–5.94		

N, total number of participants in each group; n (%), number (proportion) of participants with clinic based testing; OR, odds ratio; CI, confidence interval; NA, not applicable; <sup>1</sup>p-value for equality between categories; <sup>2</sup>p-value for interaction between gender and independent variable; <sup>3</sup>Living with relatives, in students' houses or in private accommodation; <sup>4</sup>Includes the response: 'Uncertain if any contraception was used' (girls n = 3, boys n = 10); <sup>5</sup>Includes the response: 'Uncertain if any contraception was used' (girls n = 3, boys n = 8).

girls and boys: older age, Sami/Sami-Norwegian ethnicity, residence outside the family home, higher levels of alcohol and drug use, age at first intercourse ≤ 14 years, sexual activity ≥ 2 years, ≥ 2 sexual partners past 6 months, higher number of lifetime sexual partners, age difference with last partner ≥ 1 year, and no condom use at last intercourse (Table 1). Girls in vocational classes had higher odds of clinic based testing than those with academic affiliation. No condom use at first intercourse increased the odds of clinic based testing in boys, but not in girls. Clinic based testing was not associated with school based screening or with prevalent chlamydia infection.

In multivariable analysis, the following variables increased the odds of clinic based testing in girls and boys combined: older age, age at first intercourse ≤ 14 years, no condom use at first intercourse, steady relationship, and having had higher number of lifetime partners (Table 2). No condom use at last intercourse increased the odds in girls only. Significant interaction was present between gender and ethnicity ( $p = 0.012$ ) in the multivariable model. Girls had higher odds of clinic based testing than boys, but the odds ratio varied by ethnic group. Among boys, clinic based testing varied between the

three ethnic groups with Norwegian boys having the lowest test activity. In girls, ethnic group was not associated with clinic based testing. Nagelkerke's estimate of explained variance in the multivariable model for all participants was 42%.

#### School based screening

In girls' crude analysis, academic affiliation, condom use at first intercourse, and current urogenital symptoms increased the odds of school based screening (Additional file 1), and these variables remained significant in the girls' multivariable model (Table 3). Among 243 girls reporting ≥ 1 symptom, only 10% had a positive test result. In boys, age at first intercourse ≥ 15 years and no prior treatment for chlamydia infection increased the odds of school based screening in crude analysis, while in multivariable analysis low substance use, age at first intercourse ≥ 15 years, and no condom use at last intercourse increased the odds. Assessing girls and boys combined, the following variables increased the odds of school based screening in multivariable analysis: male gender, academic affiliation, age at first intercourse ≥ 15 years, condom use at first intercourse,

**Table 2 Odds ratios for clinic based testing in multivariable logistic regression models**

Characteristic	Girls			Boys			All participants <sup>1</sup>		
	OR	95% CI	<i>p</i> <sup>2</sup>	OR	95% CI	<i>p</i> <sup>2</sup>	OR	95% CI	<i>p</i> <sup>2</sup>
<b>Gender: girls vs boys<sup>3</sup></b>									
Norwegian	NA			NA			7.96	5.26–12.04	< 0.001
Sami/Sami-Norwegian	NA			NA			3.62	1.92–6.82	< 0.001
Other	NA			NA			1.89	0.66–5.45	0.24
<b>Age</b>									
OR per year	1.47	1.20–1.81	< 0.001	1.73	1.28–2.32	< 0.001	1.54	1.30–1.83	< 0.001
<b>Family and culture</b>									
Ethnicity girls <sup>4</sup>									
Norwegian	ns			NA			1.00		0.37
Sami/Sami-Norwegian	ns			NA			1.30	0.82–2.08	
Other	ns			NA			0.78	0.38–1.60	
Ethnicity boys <sup>5</sup>									
Norwegian	NA			1.00		< 0.001	1.00		< 0.001
Sami/Sami-Norwegian	NA			2.83	1.56–5.13		2.86	1.59–5.14	
Other	NA			3.53	1.44–8.64		3.28	1.37–7.84	
<b>Sexual behaviour</b>									
Age at first intercourse									
≥15 years	1.00		0.006	1.00		0.003	1.00		< 0.001
≤14 years	1.78	1.18–2.67		2.56	1.39–4.71		2.02	1.43–2.85	
Condom use first intercourse									
Yes	ns			ns			1.00		0.013
No <sup>6</sup>	ns			ns			1.48	1.09–2.01	
Currently in a relationship									
Yes	ns			ns			1.00		0.009
No	ns			ns			0.66	0.49–0.90	
Lifetime no of sex partners									
1-2	1.00		< 0.001	1.00		< 0.001	1.00		< 0.001
3-5	2.42	1.59–3.71		4.22	1.95–9.12		3.07	2.11–4.46	
≥6	6.31	3.85–10.34		8.57	3.91–18.79		7.63	5.03–11.55	
Condom use last intercourse									
Yes	1.00		0.011	ns			ns		
No <sup>7</sup>	1.93	1.16–3.20		ns			ns		

The significant variables from stepwise selection, age and gender (if applicable) were included in the models.

OR, odds ratio; CI, confidence interval; NA, not applicable; ns, non-significant; <sup>1</sup>Interaction term between gender and ethnicity included in the model (*p* 0.012);

<sup>2</sup>*p*-value for equality between categories; <sup>3</sup>Reference group: boys; <sup>4</sup>Odds ratios in girls; <sup>5</sup>Odds ratios in boys; <sup>6</sup>Includes the response: 'Uncertain if any contraception was used' (girls *n* = 3, boys *n* = 10); <sup>7</sup>Includes the response: 'Uncertain if any contraception was used' (girls *n* = 3, boys *n* = 8).

and current urogenital symptoms. Nagelkerke's estimate in the multivariable model for all participants was 6.2% (Table 3).

The 18 girls and 11 boys with chlamydia infection and school-only test reported less condom use at both first and last intercourse, and higher number of sexual partners past 6 months and during lifetime than non-infected participants with school-only test (*p* < 0.05).

## Discussion

We found that a large proportion of adolescent girls had been tested previously. The unusually high and equal participation between genders in the school based screening and the finding of a large undetected pool of chlamydia infections confirms the value of school based testing. High school based screening and clinic based testing were associated with completely different independent

**Table 3 Odds ratios for school based screening in multivariable logistic regression models including the significant variables from stepwise selection**

Characteristic	Girls			Boys			All participants		
	OR	95% CI	<i>p</i> <sup>1</sup>	OR	95% CI	<i>p</i> <sup>1</sup>	OR	95% CI	<i>p</i> <sup>1</sup>
<b>Gender</b>									
Girls <sup>2</sup>	NA			NA			0.57	0.34–0.97	0.040
<b>Age</b>									
OR per year	0.91	0.65–1.27	0.58	0.82	0.52–1.28	0.39	0.86	0.67–1.11	0.24
<b>High school</b>									
Study affiliation									
Academic	1.00		0.001	ns			1.00		0.013
Vocational	0.33	0.17–0.65		ns			0.51	0.30–0.87	
<b>Alcohol/drug use</b>									
Low	ns			1.00		0.039	ns		
Medium	ns			0.75	0.22 to 2.58		ns		
High	ns			0.26	0.08 to 0.91		ns		
<b>Sexual behaviour</b>									
Age first intercourse									
≥15 years	ns			1.00		0.001	1.00		0.029
≤14 years	ns			0.23	0.10–0.57		0.58	0.35–0.95	
Condom use <i>first</i> intercourse									
Yes	1.00		0.010	ns			1.00		0.026
No <sup>3</sup>	0.42	0.21–0.82		ns			0.57	0.35–0.94	
Condom use <i>last</i> intercourse									
Yes	ns			1.00		0.003	ns		
No <sup>4</sup>	ns			3.86	1.59–9.41		ns		
<b>Chlamydia infection</b>									
Urogenital symptoms <sup>5</sup>									
No	1.00		<0.001	ns			1.00		0.001
Yes	4.26	1.89–9.63		ns			3.23	1.57–6.65	

Age and gender (if applicable) included in the model.

OR, odds ratio; CI, confidence interval; NA, not applicable; ns non-significant, <sup>1</sup>*p*-value for equality between categories; <sup>2</sup>Reference group: boys; <sup>3</sup>Includes the response: 'Uncertain if any contraception was used' (girls *n* = 3, boys *n* = 10); <sup>4</sup>Includes the response: 'Uncertain if any contraception was used' (girls *n* = 3, boys *n* = 8); <sup>5</sup>In girls: dysuri, vaginal discharge, intermenstrual and/or postcoital bleeding. In boys: dysuri and/or urethral discharge.

variables. To have been tested previously was mainly associated with traditional risk factors suggesting that these adolescents were aware of the behavioural determinants of chlamydia infection and thus were motivated by their own perceived risk. In contrast, school based screening mostly was associated with factors unknown to increase risk, strongly indicating the presence of other incentives. To our knowledge, this is the first study to compare clinic based testing to school based screening in a general adolescent population.

The high proportion of girls with clinic based testing is in agreement with Norwegian surveillance data and other European studies with young females accessing clinical test sites much more frequently than same-aged males [2,15,16]. The lower sexual activity among Norwegian

boys 15–20 years and young males' reluctance to access health care services may contribute to less testing [14,17]. The high participation rate in the school based screening may be explained by the following factors: thorough planning, the relevant topics, the universal offer to all students irrespective of sexual history, the 'in-class' recruitment and sampling procedures, the efficient logistics with rapid notification of positive test results, and this being the first chlamydia high school based screening in Northern Norway [18]. It is likely that invitation to participate in research increased uptake. In Norway, repeat school based studies on adolescent health and lifestyle including biological samples, have shown sustained response rates above 85% [19,20] thus suggesting a potential for sustainability of repeat school based chlamydia screening. We

observed a curious and welcoming attitude among both students and staff. Male participants frequently commented on the simplicity of urinating in a cup and the convenience of a class-wise approach with everyone getting tested. Adolescent males are more likely to accept STI testing if the testing procedures are convenient [17,21,22] and if they feel that confidentiality is maintained [23]. Individual provider characteristics may also impact on their decision [24,25]. The boys' high acceptance for school based screening challenges the notion of adolescent males as a hard-to-reach group as the selection bias normally created by low participation among boys was not observed.

### Chlamydia prevalence

High chlamydia prevalence was detected in girls irrespective of earlier test behaviour. Girls with clinic based testing had higher levels and longer duration of risk behaviours than those with school-only test. The equal infection levels may indicate effect of adherence to recommendations on testing and treatment in the first group and less adherence in the school-only test group among those with high risk behaviours. Boys with clinic based testing had approximately the same prevalence as girls. Boys with school-only test having half the prevalence of girls is consistent with less sexual activity in boys this age and thus reduced infection risk [14]. For girls and boys combined, half of chlamydia cases were detected in the school-only test group. Correspondingly these infected subjects had higher levels of risk behaviours than participants with school-only test and a negative chlamydia test result. We may have underestimated prevalence in girls as *C. trachomatis* was detected in FVU samples that are less sensitive than self-collected vaginal swabs.

### Factors associated with testing

Clinic based testing behaviour differing between ethnic groups among boys may indicate that boys' testing patterns at this age is more influenced by same-ethnicity peers and less by national recommendations. In contrast, girls' test activity was not associated with ethnicity. Early first intercourse doubled the odds of clinic based testing and was positively correlated with number of sexually active years suggesting that it may reflect a longer sexually active period with more testing opportunities. In contrast, participants who just recently started their sexual career only had limited time to seek chlamydia testing. No condom use at first and last intercourse increasing the odds of clinic based testing suggests testing for safety reasons. While condom use at any occasion is a dyadic behaviour and negotiable between partners, chlamydia testing can freely be carried out by the individual. Higher lifetime number of sexual partners being associated with increased

clinic based testing is in agreement with a study on uptake in the English National Chlamydia Screening Programme where persons being tested in the programme reported significantly higher numbers of partners than a random sample of the general population [26].

In the multivariable model using school based screening as independent outcome, current urogenital symptoms in girls and in both genders combined were the only significant traditional risk factor. However, symptoms had low positive predictive value to detect chlamydia infection which is consistent with other studies [1]. School screening reached a large proportion of adolescents at no or low risk of chlamydia infections. Among these, participants not previously tested in clinical practice may have benefited from learning the test procedure.

One-fifth of all infections were detected in participants with previous chlamydia treatment and were thus repeat infections undetected in clinical practice. This may indicate a weakness in the Norwegian testing algorithm. Our data did not allow any conclusions about the duration of an infection, or if it was transmitted by the same partner, by new sexual contacts, or was due to treatment failure or non-compliance. In addition, we had no information on time since clinic based testing. Prevalent chlamydia infection not being associated with clinic based testing indicates that adolescents do underestimate their own infection risk as also observed in other studies [27]. Although chlamydia testing in youth clinics is easily available and free of charge irrespective of the patient meeting the national test criteria or not, our study shows that a significant proportion of adolescents at risk had not been tested before the study suggesting the presence of other barriers to testing.

The strengths of this study include the representativeness achieved by high participation, the use of computer-based questionnaires to limit social desirability bias, and the use of high quality biological samples [28].

### Limitations

The study is limited by cross-sectional design that precludes establishing causality and by self-reported data on sexual behavioural and previous chlamydia treatment. The presence of some social desirability bias is likely due to the sensitive topics. Using laboratory data to assess the outcome variable 'clinic based testing' instead of a questionnaire would have improved validity. However, longer recall periods have been found accurate for assessing low-frequency events such as a previous chlamydia test [29]. In this paper, we assumed specific sexual behaviours, i.e. number of sexual partners past 6 months and circumstances related to last sexual intercourse, reported before the school screening to be representative for sexual behaviours before clinic based testing. Our assumption is based on the finding that single-events

like the most recent intercourse is valid representation of sexual behaviour over longer periods of time [30]. Previous STI test results could have influenced later sexual behaviour in the direction of less or increased risk causing a slight attenuation in the observed odds ratio estimates.

## Conclusions

More than half the girls had been tested prior to the school based screening and had high prevalence independent of clinic based testing. While clinic based testing was associated with traditional chlamydia risk factors, school based screening was mostly associated with factors unknown to increase infection risk. The high and equal participation between genders and the detection of a large chlamydia reservoir that included both first-time and repeat infections confirms the value of school based screening and suggests this approach to be further explored in Norway.

## Additional file

**Additional file 1: Sosio-demographic and sexual behaviour characteristics – prevalence and crude odds ratios for school based screening in univariable logistic regression models.**

## Abbreviations

FVU: First-void urine; STI: Sexually transmitted infection.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contribution

KG conceived and designed the study, applied for funding, collected the data, and drafted the manuscript. GSS and ASF participated in study design. KG and TW chose the main direction for data analysis and performed the statistical analyses. All authors contributed to the interpretation of the results and revised and approved the manuscript.

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**Additional file 1.** Sosio-demographic and sexual behaviour characteristics – prevalence and crude odds ratios for *school based screening* in univariable logistic regression models.

Characteristic	Girls					Boys					All participants	
	School based screening					School based screening					Interaction	
	N	n (%)	OR	95% CI	<i>p</i> <sup>1</sup>	N	n (%)	OR	95% CI	<i>p</i> <sup>1</sup>	<i>p</i> <sup>1</sup>	<i>p</i> <sup>2</sup>
<b>Total</b>	607	564 (92.9)	NA			505	470 (93.1)	NA				
<b>Age</b>												
15-16	179	168 (93.9)	1.00		0.78	149	141 (94.6)	1.00		0.72	0.51	1.00
17	193	177 (91.7)	0.72	0.33–1.61		196	180 (91.8)	0.64	0.27–1.53			
18	177	166 (93.8)	0.99	0.42–2.34		114	107 (93.9)	0.87	0.31–2.47			
19-20	58	53 (91.4)	0.69	0.23–2.09		46	42 (91.3)	0.60	0.17–2.08			
<b>Family and culture</b>												
<b>Ethnicity</b>												
Norwegian	433	402 (92.8)	1.00		0.39	353	329 (93.2)	1.00		0.88	0.62	0.39
Sami/Sami-Norwegian	131	124 (94.7)	1.47	0.59–3.18		115	106 (92.2)	0.86	0.39–1.91			
Other	43	38 (88.4)	0.58	0.22–1.60		36	34 (94.4)	1.24	0.28–5.48			
<b>Residence in school year</b>												
At home	380	356 (93.7)	1.00		0.33	314	290 (92.4)	1.00		0.42	0.86	0.15
Other <sup>3</sup>	226	207 (91.6)	0.73	0.39–1.37		191	180 (94.2)	1.35	0.65–2.83			
<b>Mothers education</b>												
≤ High school/don't know	338	312 (92.3)	1.00		0.52	334	308 (92.2)	1.00		0.31	0.26	0.69
≥ College	268	251 (93.7)	1.23	0.65–2.32		169	160 (94.7)	1.50	0.69–3.28			
<b>High school</b>												
<b>Study affiliation</b>												
Academic	323	347 (95.6)	1.00		0.002	181	171 (94.5)	1.00		0.36	0.005	0.21
Vocational	244	217 (88.9)	0.37	0.20–0.70		324	299 (92.3)	0.70	0.33–1.49			
<b>Alcohol/drug use</b>												
Low	144	133 (94.3)	1.00		0.28	140	132 (96.4)	1.00		0.11	0.046	0.71
Medium	343	322 (93.9)	0.92	0.40–2.13		212	200 (94.3)	0.63	0.22–1.83			
High	118	106 (89.8)	0.53	0.21–1.35		144	130 (90.3)	0.35	0.12–1.01			
<b>Sexual behaviour</b>												
<b>Age at first intercourse</b>												
≥ 15 years	352	330 (93.8)	1.00		0.33	311	297 (95.5)	1.00		0.014	0.018	0.22
≤ 14 years	252	231 (91.7)	0.73	0.39–1.37		172	154 (89.5)	0.40	0.20–0.83			
<b>Years sexually active</b>												
≤ 1 year	162	149 (92.0)	1.00		0.60	171	160 (93.6)	1.00		0.90	0.79	0.66
≥ 2 years	442	412 (93.2)	1.20	0.61–2.36		312	291 (93.3)	0.95	0.45–2.03			
<b>Condom use first intercourse</b>												
Yes	358	340 (95.0)	1.00		0.019	267	250 (93.6)	1.00		0.75	0.050	0.19
No <sup>4</sup>	248	223 (89.9)	0.47	0.25–0.89		226	210 (92.9)	0.89	0.44–1.81			
<b>Currently in a relationship</b>												
Yes	322	296 (91.9)	1.00		0.31	179	169 (94.4)	1.00		0.38	0.84	0.19



No	285	268 (94.0)	1.39	0.74–2.61		326	301 (92.3)	0.71	0.33–1.52			
Sex partners past 6 months												
0-1	352	323 (91.8)	1.00		0.16	273	258 (94.5)	1.00		0.33	0.65	0.097
≥ 2	248	235 (94.8)	1.62	0.83–3.19		194	179 (92.3)	0.69	0.33–1.46			
Life time no of sex partners												
1-2	206	192 (93.2)	1.00		0.96	218	206 (94.5)	1.00		0.19	0.38	0.49
3-5	191	178 (93.2)	1.00	0.46–2.18		123	117 (95.1)	1.13	0.42–3.11			
≥ 6	201	186 (92.5)	0.90	0.43–1.92		119	107 (89.9)	0.52	0.23–1.20			
<b>Last sexual partner</b>												
Age difference												
Same age or younger	144	133 (92.4)	1.00		0.79	413	386 (93.5)	1.00		0.42	0.89	0.55
Older (≥ 1 year)	445	414 (93.0)	1.11	0.54–2.26		54	52 (96.3)	1.82	0.42–7.87			
Condom use <i>last</i> intercourse												
Yes	94	90 (95.7)	1.00		0.25	168	153 (91.1)	1.00		0.10	0.72	0.059
No <sup>5</sup>	513	474 (92.4)	0.54	0.19–1.55		319	303 (95.0)	1.86	0.89–3.86			
<b>Chlamydia infection</b>												
Clinic based testing												
No	269	251 (93.3)	1.00		0.74	399	373 (93.5)	1.00		0.48	0.49	0.73
Yes	338	313 (92.6)	0.90	0.48–1.68		106	97 (91.5)	0.75	0.34–1.66			
Previous treatment												
No	485	452 (93.2)	1.00		0.59	467	438 (93.8)	1.00		0.027	0.11	0.16
Yes	122	112 (91.8)	0.82	0.39–1.71		37	31 (83.8)	0.34	0.13–0.89			
Urogenital symptoms <sup>6</sup>												
No	351	317 (90.3)	1.00		0.003	460	429 (93.3)	1.00		0.82	0.011	0.24
Yes	251	243 (96.8)	3.26	1.48–7.17		35	33 (94.3)	1.19	0.27–5.20			

N, total number of participants in each group; n (%), number (proportion) of participants with *school based screening*; OR, odds ratio; CI, confidence interval; NA: not applicable; <sup>1</sup>p-value for equality between categories; <sup>2</sup>p-value for interaction between gender and variable; <sup>3</sup>Living with relatives, in students' houses or in private accommodation; <sup>4</sup>Includes the response: 'Uncertain if any contraception was used' (girls n=3, boys n=10); <sup>5</sup>Includes the response: 'Uncertain if any contraception was used' (girls n=3, boys n=8); <sup>6</sup>In girls: dysuri, vaginal fluor, intermenstrual and/or postcoital bleeding. In boys: dysuri and/or urethral discharge



Appendix A  
Information letter  
*English translation*



## **Invitation to participate in a research project in fall 2009**

### ***‘Sexual behaviour and chlamydia among high school students in Finnmark’***

#### **Background and purpose**

This is an invitation to participate in a research study on sexual behaviour and chlamydia prevalence among high school students in Alta, Hammerfest, Sør-Varanger, Karasjok and Kautokeino. The study is conducted by the Competence Centre of Infection Control in the Regional Health Authority of North Norway, University Hospital of North Norway, and by the University of Tromsø.

Chlamydia is a common sexually transmitted infection (STI) among Norwegian adolescents. Over the years, Finnmark and Troms counties have reported higher chlamydia rates than the rest of Norway. Chlamydia infection often is asymptomatic and you can be infected without recognizing it. Untreated infection can lead to infertility, ectopic pregnancy, and chronic pelvic pain in females.

The study results will provide important knowledge on the high chlamydia rates among adolescents in Finnmark and the public health measures necessary to control infection rates.

#### **What does study participation imply?**

The participants are asked to fill in a web-based questionnaire during class hours in the presence of a teacher and two persons from the research staff. The questionnaire includes questions on future educational plans, lifestyle, attitudes towards sex, contraceptive use, and much more. It is important to answer all questions as honestly as you can. Several questions focus on personal matters. You do not have to complete this questionnaire or any question if you do not want to. It should take you about 20 minutes to fill in all answers. The questionnaire is completely confidential so nobody will see the answers you give. You will not fill in your name or personal identity number in the questionnaire.

Directly after finishing the questionnaire, you continue to the school toilet and provide a urine sample. Sampling equipment will be handed out in class. We ask all participants to provide a sample, including those who have not yet had their sexual debut.

The urine sample will be analysed at the University Hospital of North Norway in Tromsø. Participants with a positive chlamydia test result will receive a text message on their mobile phone. If we receive no reply from you, we will call you on your mobile phone. Eventually, we will send a letter by mail asking you to call us. *We will not send a letter with a positive test result to your parents’ house.*

The local health care service will provide a prescription on antibiotics free of charge to participants with a positive chlamydia test result. Sexual partners will be contacted for testing and treatment.

If approved by the Data Protection Authority and the National Regional Committee for Medical and Health Research Ethics, data on participants testing positive for chlamydia will be linked to the Norwegian Prescription Database to examine if participants did fill in the prescription for antibiotic. These data will not include personal identifiable information when received by the researchers.

***All participants will be included in a lottery with 3 persons winning an exclusive mobile phone.***

#### **Advantages and disadvantages**

You will have a free chlamydia test. If you test positive, you will receive treatment. The information in the questionnaire will provide important knowledge on chlamydia infections among adolescents in Finnmark and the public health measures needed to reduce the epidemic.

You may find some of the questions too sensitive to answer. You do not have to answer all questions.

#### **What will happen to the urine sample and the personal information?**

The data will be used for research purposes only. All the analysed data will be person non-identifiable and will not be linked to your name or your personal identity number.

We use QuestBack online survey system. The data will be safely stored on servers at the University Hospital of North Norway (UNN) in Tromsø and can only be accessed by the project manager.

Urine samples testing positive for chlamydia will be analysed using a 'finger print' technique to map the chlamydia clones present in adolescents in Finnmark and compare to international clones.

### **Voluntary participation and consent**

We must stress that your participation is voluntary. Students 16 years or older should consent to participation by filling in the questionnaire. Students younger than 16 years have to provide written consent signed by their parents or guardians. You may withdraw from the study at any time and without stating any reason.

Students choosing not to participate can do school work while the others are filling in the questionnaire. School attendance is mandatory while the study is conducted. Absence will be registered by the teacher.

Please, contact us if you choose to withdraw from the study or if you have any questions: Project manager Kirsten Gravningen, phone: 776 27044/ email: [kirsten.gravningen@unn.no](mailto:kirsten.gravningen@unn.no) or public health nurse/ research assistant Randi E. Olsen, phone: 776 69552/ [randi.elisabeth.olsen@unn.no](mailto:randi.elisabeth.olsen@unn.no).

### **Privacy policy and security**

All information given in the questionnaire is strictly confidential. The staff is bound by confidentiality. UNN Tromsø by the Managing Director is responsible for data management. The data management and security system is approved by the Data Protection Official at UNN.

### **Bio-bank**

Urine samples with a negative chlamydia test result are thrown after two days. Samples with a positive test result will be stored until the 'finger print' analysis is complete and then be thrown away. If you consent to participate in the study, you also consent to temporal storage of a chlamydia positive urine sample until January 2010.

### **The right to access and delete personal data and the deletion of samples**

If you consent to participation, you have the right to view your personal information, and errors can be corrected. If you withdraw from the study, you can request deletion of the collected data unless the data already are included in analyses or in scientific publications.

### **Economy**

The study is funded by Sparebank1 Nord-Norges Medical Research Grant, The Norwegian Directorate of Health, and The North Norway Regional Health Authority.

### **Information about the study results**

The study results will be published at research conferences/ meetings and in scientific articles based on larger groups, and will not be stratified by class or school.

### **Ethics**

The study is approved by the National Regional Committee for Medical and Health Research Ethics.

### **Duty of confidentiality**

If you are younger than 16 years and have a positive chlamydia test result, we will contact only you for treatment and your parents/guardians will not be informed. The information in the questionnaire is subject to duty of confidentiality towards the parents according to The Norwegian Health Research Act §17-4.

**Consent to study participation**

*Students younger than 16 years, have to provide written consent from the parents or guardians to participate in the research study.*

I hereby consent to my daughter/ son ..... (name) participating in the study.

.....

(Signed by parents/guardians, date)



**Appendix B**  
Information letter  
*Original Norwegian version*





## **Forespørsel om deltakelse i forskningsprosjektet høsten 2009** ***”Seksualitet og klamydia blant elever i videregående skole i Finnmark”***

### **Bakgrunn og hensikt**

Det er forespørsel om å delta i en studie som skal undersøke seksualvaner og forekomst av klamydia blant elever i videregående skole i Alta, Hammerfest, Sør-Varanger, Karasjok og Kautokeino. Studien utgår fra Kompetansesenter i smittevern Helse Nord, Universitetssykehuset i Nord-Norge og Universitetet i Tromsø.

Klamydia er en seksuelt overført infeksjon (kjønns sykdom) som er svært vanlig blant norsk ungdom. Finnmark og Troms har gjennom årene rapportert høyere forekomst av klamydia enn resten av landet. Ofte gir klamydia få plager, og det gjør at man kan være smittet med klamydia uten å vite det. Ubehandlet klamydiainfeksjon kan seinere i livet gi kvinner komplikasjoner som barnløshet, svangerskap utenfor livmoren og smerter i bekkenet.

Resultatene fra denne studien vil gi viktig informasjon om bakgrunnen for den høye forekomsten av klamydia blant ungdom i Finnmark, og hvordan helsetjenesten kan styrke det forebyggende arbeidet mot klamydia. Studien gjennomføres i skolene høsten 2009.

### **Hva innebærer studien?**

Deltakerne besvarer et web-basert spørreskjema i klasserommet i skoletida. Læreren og to personer fra prosjektet vil være til stede. Spørreundersøkelsen dreier seg om fremtidsplaner, livsstil, holdninger til sex, prevensjonsbruk, med mer. Det er viktig at man besvarer spørsmålene så ærlig som mulig. Flere spørsmål handler om personlige forhold. Er det spørsmål man ikke kan eller vil svare på, lar man være. Det tar maks. 20 minutter å besvare skjemaet. Alle svarene er konfidensielle. Ingen lærere eller andre med tilknytning til skolen får se skjemaet etter at det er fylt ut. Man skal ikke fylle ut navn eller fødselsnummer på skjemaet.

Rett etter besvaring av skjema, går man til skolens toalett og avgir en urinprøve. Prøvetakingsutstyr deles ut i klassen. Av forskningsmessige hensyn er det viktig at alle deltakerne leverer urinprøve, også elever som ikke har debutert seksuelt.

Urinprøvene undersøkes for klamydia ved Universitetssykehuset i Tromsø. Funn av klamydia varsles til deltakeren *via sms på mobil*. Dersom vi ikke får kontakt via sms, vil vi ringe på mobilen. Siste utvei er å sende brev i nøytral konvolutt hvor vi ber om å bli kontaktet på et oppgitt telefonnummer. *Brev med positiv klamydiaprøve vil ikke sendes hjem til foreldrene.*

Lokal helsetjeneste vil sørge for behandling av klamydia ved å skrive resept på én-gangsdose med antibiotika. Behandlingen er gratis. Det vil gjøres smitteoppsporing blant partnere for å sikre at partnerne får tilbud om behandling.

Med forbehold om godkjenning fra Datatilsynet og Regional komité for medisinsk og helsefaglig forskningsetikk (REK nord), vil data om deltakere som tester positivt for klamydia, bli koblet mot opplysninger i Nasjonalt reseptregister for å undersøke om de har innløst resept på antibiotika fra apoteket. Data om deltakerne som har hentet ut antibiotika, vil utleveres til forsker uten person-identifiserende kjennetegn, bare som opplysninger om kjønn, alder og utleveringsdato for antibiotika.

***Deltakerne i studien er med i loddrekning om 3 fine mobiltelefoner i desember 2009.***

### **Mulige fordeler og ulemper**

En fordel med å delta er at du vil bli testet for klamydia og at du vil få nødvendig behandling hvis prøven er positiv. Opplysningene på spørreskjemaet vil bidra til å øke kunnskapen om den høye forekomsten av klamydia blant ungdom i Finnmark, og hvilke tiltak som kan settes inn for å forebygge og hindre klamydiaepidemien.

Undersøkelsen inneholder enkelte spørsmål som for noen deltakere kan oppleves som sensitive. Du trenger ikke å besvare alle spørsmålene i skjemaet.

## **Hva skjer med prøvene og informasjonen om deg?**

Informasjonen som registreres, skal kun brukes slik som beskrevet i hensikten med studien. All informasjon vil bli behandlet uten navn, fødselsnummer eller direkte gjenkjenning opplysninger.

Prosjektet benytter Questback i gjennomføringen av spørreundersøkelsen. Svarene lagres på sikre servere Universitetssykehuset Nord-Norge (UNN) i Tromsø hvor kun prosjektleder har tilgang.

Urinprøver som er positive for klamydia, vil bli undersøkt nærmere med ”fingeravtrykksanalyse” for å kartlegge hvilke kloner av klamydia som fins blant ungdom i Finnmark, og om det er de samme klonene som ellers i Norden.

## **Frivillig deltakelse og samtykke**

Deltakelse i studien er frivillig. Elever som er fylt 16 år, vil gjennom å besvare spørreskjemaet gi sitt samtykke til å delta. Elever som *ikke har fylt 16 år*, må levere skriftlige samtykke fra foreldrene. Man kan når som helst og uten å oppgi grunn, trekke seg fra studien uten at dette får noen konsekvenser.

Elever som ikke deltar i studien, kan gjøre skolearbeid i klasserommet mens de andre besvarer spørreskjemaet. Det er *obligatorisk frammøte* til timen, og det føres fravær.

Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte:

Prosjektleder Kirsten Gravningen, tlf dagtid: 776 27044/e-post til: [kirsten.gravningen@unn.no](mailto:kirsten.gravningen@unn.no).  
Helsesøster/forskningsass. Randi E. Olsen, tlf dagtid 776 69552/ [randi.elisabeth.olsen@unn.no](mailto:randi.elisabeth.olsen@unn.no).

## **Personvern og sikkerhet**

Alle opplysninger som gis i spørreskjemaet, er konfidensielle. Ansatte i prosjektet har taushetsplikt. Universitetssykehuset Nord-Norge Tromsø ved administrerende direktør er databehandlingsansvarlig.

Datahåndtering og datasikkerhet er godkjent av Personvernsombudet ved UNN HF.

## **Biobank**

Urinprøver som er negative for klamydia, kastes etter to dager. Urinprøver som er positive for klamydia, vil bli lagret inntil ”fingeravtrykksanalyse” er utført. Deretter kastes også disse urinprøvene.

Hvis man sier ja til å delta i studien, gir man også samtykke til at urinprøven (hvis den er klamydiapositiv) og analyseresultatet oppbevares midlertidig til januar 2010.

## **Rett til innsyn og sletting av opplysninger om deg og sletting av prøver**

Hvis man sier ja til deltakelse, har man rett til innsyn i egne registrerte opplysninger, og eventuelle feil kan korrigeres. Dersom man trekker seg fra studien, kan man kreve å få slettet innsamlet informasjon, med mindre denne allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

## **Økonomi**

Studien og biobanken er finansiert gjennom forskningsmidler fra Sparebank1 Nord-Norges Medisinske forskningspris 2008, Helsedirektoratet og fra Kompetansesenter i smittevern Helse Nord.

## **Informasjon om utfallet av studien**

Resultater fra studien vil kunne publiseres som gruppedata på faglige konferanser/ møter og i vitenskapelige artikler uten at den enkelte klasse eller skole kan gjenkjennes. Resultatene vil brukes som grunnlag for å styrke tiltak mot seksuelt overførte infeksjoner blant ungdom i Finnmark.

## **Etisk godkjenning**

Prosjektet er godkjent av REK nord.

## **Taushetsplikt**

Dersom deltakere under 16 år får påvist klamydia, vil han/hun kontaktes direkte for behandling.

Foreldrene til deltakere under 16 år vil *ikke informeres* om prøveresultatet. Prosjektet har også taushetsplikt overfor foreldre/ foresatte vedrørende opplysninger som eleven avgir i spørreskjemaet. Ovenstående er i henhold til helseforskningsloven § 17, 4. ledd.

# Samtykke til deltakelse i studien

*Elever som enda ikke er fylt 16 år, må levere skriftlig samtykke fra foreldrene til å delta i studien.*

Jeg samtykker til at mitt barn .....(navn) deltar i studien

-----  
(Signert av foreldre/foresatte, dato)

**NB: Elever under 16 år må ta utskrift av dette dokumentet og levere underskrevet samtykke til prosjektleder samme dag som studien gjennomføres i klassen.**



Appendix C  
Information letter  
*Original Sami version*



## **Jearaldat searvat dutkanprošektii 2009 čavčča** **”Seksualitehta ja klamydia Finnmárkku joatkkaskuvlaohppiid gaskka”**

### **Duogáš ja ulbmil**

Dá lea jearaldat searvat iskkadeapmái mas mii galgat iskkadit Álttá, Hammerfeastta, Mátta-Várjjaga, Kárášjoga ja Guovdageainnu joatkkaskuvlaohppiid seksuála dábiid ja lea go sis klamydia. Iskkadeami čađahit *Kompetansesenter i smittevern Helse Nord* (Davvi Dearvvašvuoda njoammuneastadeami Gelbbolašvuodaguovddáš), Davvi-Norgga Universitehtabuohcciviessu ja Romssa Universitehta.

Klamydia lea seksuálalaččat njommon infekšuvdna (nuoskkesdávda), mii lea ollu nuorain Norggas. Finnmárkkus ja Romssas lea dát dávda mañemus jagiid gávdnon sakka eanet go muđui riikkas. Dávjá olmmoš ii dovdda ahte sus lea klamydia, ja dat dahká ahte olbmossáhtá leat dávda vaikko ieš ii dieđe ge. Jus ii dálkkot klamydia, de sáhtá dat dagahit nissonolbmui mañjelis eallimis ahte ii sáhte oažžut máná, dahje ahte ohki (máná) šaddagoahtá olggobeallái mánágoađi, ja sáhtá maid dagahit bákčasiid vuolil.

Dán iskkadeami bohtosat addet deatalaš dieđuid dasa manne nu ollu Finnmárkku nuorain lea klamydia, ja movt dearvvašvuodabálvalus sáhtá hehttet klamydia leavvamis. Iskkadeapmi čađahuvvo skuvllain 2009 čavčča.

### **Máid sisttisoallá iskkadeapmi?**

Oasseváldit vástidit web-vuodustuvvon jearahallanskovi luohkkálanjas skuvlaáiggis. Oahpaheaddji ja guokte olbmo proševttas leat doppe dalle. Jearahallanskovis leat jearaldagat boahhteáiggi plánaid birra, eallinvuogi ja dan birra makkár oainnut dus leat sexii, prevenšuvnnaid geavaheapmái jna. Lea deatalaš vástidit gažaldagaid nu rehálaččat go vejolaš. Mánja gažaldaga leat persovnnalaš dilálašvuodaid birra. Jus leat gažaldagat maid it hálit vástidit, de lea dat ortnegis. Ádjána eanemusat 20 minuhta vástidit jearaldagaid. Buot vástádusat dollojuvvojit čiegusin. Ii oktage oahpaheaddji, eai ge earát geat gullet skuvlii, beasa oaidnit vástádusaid. Skovváii ii galgga čállit nama, ii ge riegeádanbeaivvi.

Dakkaviđe go leat vástidan skovi, galggat mannat skuvlla hivssegii ja addit gožžaiskosa. Mii addit luohkkálanjas doasa masa goččat. Dutkama dihte lea dehálaš ahte buot oasseváldit addet gožžaiskosa, maiddáii sii geain ii leat vuos leamaš sexa.

Romssa Universitehta iská gožžaiskosiin lea go klamydia. Jus gávnnavuvvo klamydia, de mii dieđihit *sms bokte mobiltelefonidnii*. Jus eat oaččo oktavuoda sms bokte, de riŋget mobiltelefonidnii. Mañemus vejolašvuotta lea sáddet reivve nøytrála konvoluhtas, mas mii bivdit sáddet telefonnummára. *Reive mii dieđiha ahte dus lea klamydia, ii sáddejuvvo ruoktot du váhnemiidda.*

Báikkálaš dearvvašvuodabálvalus addá divššu klamydia vuostá dan bokte ahte čállá resepta antibiotika-dálkasii maid galggat okte váldit. Dikšu lea nuvttá. Mii fertet de diehtit geainna/geaiguin son, geas gávnnavuvvo klamydia, lea ovtastallan, vai beassat maiddáii su/sin dikšut.

Jus Databearráigeahčču ja medisiinnalaš ja dearvvašvuodafágalaš dutkanetihkka (REK nord) dohkkehit, de datat oasseváldiid birra geain lea klamydia, čadnojuvvojit dieđuide Našuvnnalaš reseptaregistarii iskan dihte leat go sii viežžan antibiotika apotehkas. Diehtu geat vižžet antibiotika resepttain maid leat ožžon, sáddejuvvo dutkiide almmá nama haga ja eará dieđuid haga mat sáhtáše gávdnat geat sii leat. Dutkit ožžot dušše dieđuid agi ja sohka beali birra ja guđe dáhtona lea viežžan antibiotika-dálkasa.

***Sii geat servet iskkadeapmái leat mielde vuorbádeamis, mas geassit golbma fiinna mobiltelefonna juovlamánus 2009.***

### **Vejolaš ovdamunit ja heajut bealit**

Okta ovdamunni lea ahte beasat iskkahit lea go dus klamydia ja ahte oaččut dárbblaš divššu jus dus lea. Dieđut maid mii oažžut jearahallanskovi bokte veahkehit min oahppat eambo dan birra manne nu ollu Finnmárkku nuorain lea klamydia, ja movt sáhtášii hehttet klamydia leavvamis ja movt hehttet klamydia-epidemiija.

Iskkadeamis leat gažaldagat mat soitet soames ohppiid mielas leame menddo persovnnalaččat. Don it dárbbáš vástidit buot gažaldagaid, jus it hálit.

### **Mii geavvá iskosiiguin ja dieđuiguin du birra?**

Dieđut maid registreret, galget adnot dušše nu movt lea čilgejuvvon iskkadeami ulbmilis. Dieđut eai sáhte čadnot nammii, riegádannummarii eai ge eará dieđuide mat sáhttet mitalit gii lea máid vástidan.

Prošeakta geavaha Questback jearahallaniskadeami čađaheamis. Vástádusat vurkejuvvojit Davvi-Norgga Buohcciviesu (UNN) sihkkaris serváriidda, gosa dušše prošeaktajodiheaddji beassá.

Gožžaiskosat main gávdno klamydia, iskkaduvvojit dárkileappot nu gohčoduvvon “suorbmaluoddaanalysa” bokte, gávnahan dihte makkár klamydia-klonat Finnmárkku nuorain leat, ja leat go dat seammaláganat go muđui Davviriikkain.

### **Eaktudáhtolaš searvan ja miediheapmi**

Iskkadeapmái searvan lea eaktudáhtolaš. Oahppit geat leat deavdán 16 jagi, miedihit searvamii dan bokte ahte vástidit gažaldagaid. Oahppit geat *eai leat deavdán 16 jagi*, fertejit addit váhnemiid vuolláičállaga. Sáhtát vaikko goas geassádit iskkadeamis, it ge dárbbáš mitalit manne, ii ge das leat mihkke váikkuhusaid dutnje.

Oahppit geat eai searvva iskkadeapmái sáhttet bargat skuvlabargguid luohkkálanjas dan botta go earát vástidit jearaldagaide. Lea *bákkolaš boahit diibmui*, ja jávkan merkejuvvo.

Jus mañnil háliidat geassádit dahje jus leat gažaldagat iskkadeami birra, de sáhtát váldit oktavuoda: Prošeaktajodiheaddji Kirsten Gravningen, tlf beaivet: 776 27044/e-poasta:

[kirsten.gravningen@unn.no](mailto:kirsten.gravningen@unn.no).

Dearvvašvuodadivššár/dutkanassisteanta Randi E. Olsen, tlf beaivet 776 69552/

[randi.elisabeth.olsen@unn.no](mailto:randi.elisabeth.olsen@unn.no).

### **Persovdnasuodjalus ja sihkarvuolta**

Buot dieđut mat addojuvvojit jearahallanskovis dollojuvvojit čiegusin. Prošeavtta bargiin lea jávohisvuodageasku. Davvi-Norgga Buohcciviesu Romssas, hálddhusdirektora bokte, lea dat geas lea ovddasvástáduš gieđahallat dieđuid.

Dieđuid gieđahallama ja datasihkarvuoda lea UNN HF Persovdnasuodjalusáittardeaddji dohkkehan.

### **Biobánku**

Gožžaiskosat main ii leat klamydia, bálkestuvvojit 2 beavvi mañnel. Gožžaiskosat main lea klamydia, vurkejuvvojit dassáži go “suorbmaluoddaanalysa” lea čadahuvvon. Dasto bálkestuvvojit maiddái dát gožžaiskosat.

Jus miedihat searvat iskkadeapmái, de seammás dohkkehat ahte gožžaiskkus (jus das lea klamydia) ja analysaboadus vurkkoduvvo gitta ođđajagemánu 2010 rádjái.

### **Vuoigatvuolta diehtit ja sihkuhit dieđuid iežat birra ja iskosiid duššindahkat**

Jus miedihat searvat iskkadeapmái, de dus lea maid vuoigatvuolta oažžut diehtit makkár dieđut leat registrerejuvvon du birra ja vejolaš boasttuvuodaid sáhtát njulget. Jus geassádat iskkadeamis, de sáhtát gáibidit ahte čohkkejuvvon dieđut du birra sihkkajuovvojit, jus dat juo eai leača oassin analysain dahje geavahuvvon dieđalaš čállošin.



### **Ekonomiija**

Iskkadeapmi ja biobánku leat ruhtaduvvon dutkanruđaid bokte maid Seastinbánku 1 Davvi-Norga lea juolludan Medisiinnalaš 2008 dutkanbálkášumi bokte, ja maidđái Dearvvašvuodadirektoráhtta ja *Kompetansesenter i smittevern Helse Nord* (Davvi Dearvvašvuoda njoammuneastadeami Gelbbolašvuodaguovddáš) leat ruđalaččat dorjon proševtta.

### **Diedut iskkadeami bohtosa birra**

Iskkadeami bohtosiid sáhtta almmuhit oppalaš diehtun fágalaš konferánsain/čoahkkimiin ja dieđalaš artihkkaliin, nu ahte ii sáhte dovdat ovttaskas skuvlla dahje luohká. Bohtosiid sáhtta atnit nannet doaimmaid seksuála dávdmaid njoammuma vuostá Finnmárkku nuoraid guovdu.

### **Ehtalaš dohkkeheapmi**

REK nord lea dohkkehan proševtta.

### **Jávohisvuoda doallan**

Jus vuollel 16 jagi oasseváldis gávnnahuvvo klamydia, de váldit suinna njuolga oktavuoda divššu birra. Vuollel 16 jahkásaš oasseváldiid váhnemiidda *ii dieđihuvvo* iskkadeami boadus. Váhnemat/fuolaheaddjit eai ge oaččo diehtit maide eará das maid oahppi vástida jearahallanskovis. Dát lea dearvvašvuodadutkama lága § 17, 4. laddasa mielde.

## **Miediheapmi searvat iskkadeapmái**

*Oahppit geat eai leat vuos deavdán 16 jagi, fertejit addit skovi, masa váhnemat leat mieđihan ahte searvvat iskkadeapmái.*

Mun suovan iežan máná .....(namma) searvat iskkadeapmái

-----  
(Váhnemiid/fuolaheddjiid vuolláičála, beaivi)

**NB: Oahppit vuollel 16 jagi fertejit prentet dán dokumeantta ja addit prošektajodiheaddjái vuolláičallojuvnon skovi seamma beaivvi go iskkadeapmi čadahuvvo skuvllas.**



Appendix D  
Questionnaire  
*English translation*



## Research study: Sexual behaviour and chlamydia in Finnmark

**First, we'd like to have some information  
about your background and how you look at  
yourself**

---

**1) \* Gender**


Boy/man  Girl/woman

**2) \* Year of birth, four digits (YYYY):**

**3) \* School municipality**

Select answer

**4) Where do you live (residence) during the school year?**

- At home with my parents/guardians
  - Grandparents/other relatives
  - Private room/apartment
  - Student house
  - Host family
  - Other
- 

## Education

---

### 5) What is the highest level of education you plan to complete?

- High school (academic affiliation, sport, music-dance-drama)
- Vocational school
- College or university  $\leq 4$  years (ex: bachelor, teacher, police, nurse, engineer, journalist)
- College or university  $> 4$  years (ex: master's degree, lawyer, civil engineer, doctor, dentist)
- I have not decided yet
- Other

### 6) What level of education did your mother complete?

- 9 (7) years of elementary school
- Vocational school
- High school degree
- College or university  $\leq 4$  years
- College or university  $> 4$  years
- Don't know

### 7) What level of education did your father complete?

- 9 (7) years of elementary school
- Vocational school
- High school degree
- College or university  $\leq 4$  years
- College or university  $> 4$  years
- Don't know



## Culture and contact

---

**8) My perceived ethnicity is: (Tick all options that apply)**

Norweg.  Sami  Russian  Kven  Finnish  Other

**9) Ethnicity of grandparents, mothers' side: (Tick all that apply)**

Norweg.  Sami  Russian  Kven  Finnish  Other

**10) Ethnicity of grandparents, father's side: (Tick all that apply)**

Norweg.  Sami  Russian  Kven  Finnish  Other

**11) Do you and your parents/guardians have a religious affiliation?**

	Church of Norway	Laestadian	Jehovas witnesses/ Pentecostals	Russian orthodox church	Islam	No affiliation
Myself	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Mother	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Father	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>



## Self-Esteem

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*The following questions address whether you have negative thoughts about yourself. For each statement, please tick the most suitable category.*

**12) Thinking negatively about myself is something ...**

	Strongly agree	Agree	Don't know	Disagree	Strongly disagree
I often do	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
I automatically do	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
that feels normal to me	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
that is typical for me	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
I find difficult not to do	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
I will start before I realize that I'm actually doing it	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>




## Internet

---

**13) What do you do on the Internet that is related to love and sexuality: (You may tick more than one)**

- I look for someone to have a flirt with
- I look for a sweetheart/partner
- I read erotic texts (short stories/stories)
- I watch erotic pictures/movies
- I watch pornographic pictures/movies
- I check out dating sites
- I respond to sexual contact ads
- I chat with peers
- I seek sex education/advice
- I buy sex products (video, devices, etc.)
- I contact prostitutes
- I do nothing related to love or sexuality on the Internet

**14) Have you ever met someone on the Internet that you later met off-line and had sex with in real life?**

- Yes
  - No
- 

*If the respondent ticks 'YES' on the question above, question 15-16 will appear on the screen:*

**15) How many times have you met someone on the Internet that you later had sex with in real life? (enter the number using 2 digits)**

**16) What was the purpose of meeting the person you met and had sex with in real life (last time it happened?)**

- To start a romantic relationship
- To start a sexual relationship
- To have sex only once
- To have sex outside my steady relationship



## Substance use

**17) Have you ever tried or do you use any of the following substances? (Please, tick one on each line)**

	Never tried	Tried	Occasional use	Regular use
Snuff (smokeless tobacco)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cigarettes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alcohol	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cannabis (hashish, marijuana)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Amphetamine (speed)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Ecstasy (E)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



**18) Your feelings about sexuality... (Tick one on each line)**

	Strongly agree	Agree	Don't know	Disagree	Strongly disagree
I would really feel nervous if I started a sexual relationship with someone	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sexual fantasies are healthy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I really like the idea of being touched sexually	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I really like the idea of me touching someone sexually	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**19) Do you currently have a girl(boy)friend?**

- No, I've never had a girl(boy)friend
- I had previously, but not now
- Yes, I have one now



*If the respondent ticks 'YES' on question 19, question 20 will appear on the screen:*

**20) If you currently have a girl/boyfriend, what gender is she/he?**

Boy/male  Girl/female



*If the respondent ticks 'I had one previously' on question 19, question 21 will appear on the screen:*

**21) If you previously had a girl/boyfriend, what gender was she/he?**

Gutt/mann  Jente/kvinne



---

### **Contraception and chlamydia infections**

---

**22) During the past year have you done any of the following?**

	Yes	No
Bought condoms?	<input type="checkbox"/>	<input type="checkbox"/>
Received free condoms from the school nurse, the youth clinic or others?	<input type="checkbox"/>	<input type="checkbox"/>
Practiced putting on condom?	<input type="checkbox"/>	<input type="checkbox"/>

**23) Have you visited the school nurse, the youth clinic or a medical center to get any of the following during the past 2 years?**

	Yes	No
Condoms	<input type="checkbox"/>	<input type="checkbox"/>
Other contraception	<input type="checkbox"/>	<input type="checkbox"/>
Advice/testing because you/your partner suspected pregnancy	<input type="checkbox"/>	<input type="checkbox"/>
Advice for sexually transmitted infections (chlamydia, herpes, HIV, etc.)	<input type="checkbox"/>	<input type="checkbox"/>
Testing or treatment for sexually transmitted infections (chlamydia, herpes, HIV, etc.)	<input type="checkbox"/>	<input type="checkbox"/>



## Chlamydia testing and risk-assessment

---

### 24) How did you react to being chlamydia tested at school?

	Strongly agree	Agree	Don't know	Disagree	Strongly disagree
I was glad to be offered a test	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I reacted negatively to the offer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
It did not affect me positively or negatively	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### 25) How do you assess your own risk of being infected by chlamydia?

- No risk
- Low risk
- Medium risk
- High risk
- Very high risk
- Don't know



*If the respondent ticks 'No risk' or 'Low risk' on question 25, question 26 will appear on the screen:*

### 26) The reason (-s) why you answered no or low risk is: (You may tick more than one)

- I never have sex with anyone
- I have a steady partner
- I trust my partner to inform about an infection
- I can assess if my partner has a chlamydia infection
- I always use a condom
- Other reasons



## Chlamydia infection

---

**27) Have you previously been tested for chlamydia?**

- Yes, once
- Yes, twice
- Yes, 3 times
- Yes, 4 times or more
- No

**28) Have you previously been treated for chlamydia infection?**

- Yes, once
- Yes, twice
- Yes, 3 times
- Yes, 4 times or more
- No



*If the respondent ticks 'Yes, once, twice, 3 times or  $\geq$  4 times' on question 28, question 29 will appear on the screen:*

**29) The last time you were treated for chlamydia, was (were) your partner (-s) notified for treatment?**

- Yes  No  Don't know



*If the respondent ticks 'Yes' on question 29, question 30 will appear on the screen:*

**30) The last time you were treated for chlamydia infection, who notified your partner (-s)?**

- I did it myself
- The school nurse/public health nurse
- The physician
- Other



**31) In the past 4 weeks, have you received antibiotic treatment for chlamydia or another infection?**

Yes  No  Don't know



*If the respondent ticks 'Girl' on Gender, question 32 will appear:*

**32) Girls: Do you currently have any of the following symptoms? (Place a tick on each line)**

	Yes	No
Burning sensation/pain on urination	<input type="checkbox"/>	<input type="checkbox"/>
Changed or increased vaginal discharge	<input type="checkbox"/>	<input type="checkbox"/>
Lower abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>
Vaginal bleeding between periods	<input type="checkbox"/>	<input type="checkbox"/>
Vaginal bleeding after sex	<input type="checkbox"/>	<input type="checkbox"/>



*If the respondent ticks 'Boy' on Gender, question 33 will appear:*

**33) Boys: Do you currently have any of the following symptoms? (Place a tick on each line)**

	Yes	No
Pain/burning sensation on urination	<input type="checkbox"/>	<input type="checkbox"/>
Urethral discharge	<input type="checkbox"/>	<input type="checkbox"/>
Testicular swelling or tenderness	<input type="checkbox"/>	<input type="checkbox"/>
Rash/itching/soreness on the penis head	<input type="checkbox"/>	<input type="checkbox"/>



## Sexual orientation

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**34) What is your sexual orientation?**

- Heterosexual (straight)  
 Homosexual (gay/lesbian), bisexual  
 Not sure

**35) Have you ever had homosexual experiences?**

Yes  No



**36) Have you ever had sexual intercourse?**

Yes  No



*If the respondent ticks 'Girl' on Gender and 'Yes' on question 36, question 37 will appear:*

**37) Have you ever done any of the following together with a boy?**

	Ja	Nei
Hugged and held arms around each other	<input type="checkbox"/>	<input type="checkbox"/>
Kissed	<input type="checkbox"/>	<input type="checkbox"/>
Kissed with tongues	<input type="checkbox"/>	<input type="checkbox"/>
Been touched all over your body by the partner	<input type="checkbox"/>	<input type="checkbox"/>
Touched the partner all over his body	<input type="checkbox"/>	<input type="checkbox"/>



*If the respondent ticks 'Boy' on Gender and 'Yes' on question 36, question 38 will appear:*

**38) Have you ever done any of the following together with a girl?**

	Yes	No
Hugged and held arms around each other?	<input type="checkbox"/>	<input type="checkbox"/>
Kissed	<input type="checkbox"/>	<input type="checkbox"/>
Kissed with tongues	<input type="checkbox"/>	<input type="checkbox"/>
Been touched all over your body by the partner	<input type="checkbox"/>	<input type="checkbox"/>
Touched the partner all over her body	<input type="checkbox"/>	<input type="checkbox"/>



*If the respondent ticks 'No' on question 36, questions 39-43 will appear:*

**39) Have you ever wanted to have sexual intercourse?**

No  Yes, occasionally  Yes, often

**40) Have you ever started a sexual intercourse?**

No  Yes



**41) There may be many reasons for you not having had sexual intercourse. Please highlight the main reasons for not having had sex (You may tick more than one)**

- I'm not ready to have sex yet
- I'm too shy
- I'm not interested of sex
- I'm waiting for the right person
- I've got to be in love
- I'll wait until I get married
- I've not had the opportunity
- I think it is wrong/immoral
- My partner does/did not want to
- I'm scared of getting pregnant/my partner getting pregnant
- I'm afraid that my parents would disapporove
- None of my friends have had intercourse yet
- I'm afraid that it will hurt
- Other reasons

**42) From the list above, what is the main reason you have not had sexual intercourse yet?**

Select answer



**43) Please, consider if the following statements about sexuality and sexually transmitted infections are right or wrong.**

	Correct	Wrong	Don't know
A woman can get pregnant without the male ejaculating	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
A woman's chance of getting pregnant increases if she has an orgasm	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Menstrual bleeding indicates physical maturation in women	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chlamydia is sexually transmitted and can infect both women and men	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Boys cannot have erection before they reach puberty	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
To get pregnant a women needs to have sex with the male partner more than once	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Egg cells origin in the uterus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Masturbation is harmless	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
'Safe periods' are as safe as birth control pills to prevent pregnancy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gonorrhoea is the most common sexually transmitted infection in Norway	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



*If the respondent ticks 'Yes' on question 36, questions 44-49 will appear:*

---

### **First sexual intercourse**

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**44) How old were you at your first intercourse? (Please, indicate age in two digits, eg. 17)**

**45) At your first intercourse, how old was your partner? (Please, indicate age in two digits, eg. 17)**



**46) At your first intercourse, how long had you known your partner?**

- Had met him/her for the first time the same day/evening
- Less than a week
- 1-4 weeks
- 1-6 months
- More than 6 months

**47) Did you use alcohol/drugs in connection with your first intercourse?**

- No
- Yes, but I was not affected by it
- Yes, I was a little tipsy
- Yes, I was very drunk/drugged up



**48) There are usually many different reasons for a person to have sexual intercourse. What was your reason to have your first sexual intercourse? (Tick one on each line)**

	Yes	No
Because I was in love with my partner	<input type="checkbox"/>	<input type="checkbox"/>
Because my partner wanted to	<input type="checkbox"/>	<input type="checkbox"/>
Because everyone else had done it	<input type="checkbox"/>	<input type="checkbox"/>
Because my partner used pressure	<input type="checkbox"/>	<input type="checkbox"/>
Curiosity/excitement	<input type="checkbox"/>	<input type="checkbox"/>
I was sexually aroused/horny	<input type="checkbox"/>	<input type="checkbox"/>
To gain experience	<input type="checkbox"/>	<input type="checkbox"/>
Don't know, it just turned out that way	<input type="checkbox"/>	<input type="checkbox"/>

**49) Did you use any kind of contraception at your first intercourse?**

- No
- Yes, condoms
- Hormonal contraception (the pill, p-patch, p-injections, etc)
- IUD
- Both condoms and other contraception
- Emergency pill
- Other protection
- Withdrawal (coitus interruptus)
- Don't know



*If the respondent ticks 'Yes' on question 36 and 'No, Hormonal contraception, IUD, Emergency pill, Other, Withdrawal or Don't know' on question 49, question 50 will appear:*

**50) What were your reasons for not using a condom at first intercourse? (Please tick all reasons that apply)**

- Had no condoms available
- I was trying for a baby
- I was not worried of sexually transmitted infections
- I was unprepared
- Too drunk/ drugged up
- I would not risk losing my erection
- Dared not suggest a condom
- It feels better without
- Not sure how to put it on
- Used other methods of contraception (the pill, IUD, etc)
- Used other methods of contraception (withdrawal, safe periods)
- Other



*If the respondent ticks 'Yes' on question 36, question 51 will appear:*

**51) Have you had sexual intercourse more than once?**

Yes  No



*If the respondent ticks 'Yes' on question 36 and 'Yes' on 51, questions 52-55 will appear:*

**52) How many sexual partners have you had the last 6 months? (Use two digits, eg. 02)**

**53) How many sexual partners have you had past 12 months? (Use two digits, eg. 02)**

**54) What is your lifetime number of sexual partners, ie total number of partners? (Use two digits, eg. 02)**



**55) How long time has it been since your last sexual intercourse?**

- Less than a week
- 1-4 weeks
- 1 month - less than 3 months
- 3 months - 1 year
- More than 1 year ago



*If the respondent ticks 'Yes' on question 36 and 'Less than a week' or '1-4 weeks' on 55, question 56 will appear:*

**56) Approximately how many times have you had sexual intercourse the past month?**

- Once
- 2-5
- 6-9
- 10-30
- More than 30



*If the respondent ticks 'Yes' on question 36, question 57 will appear.*

## Questions about the *first time* you had intercourse with your *last* sexual partner

---

**57) Did you use a condom the first time you had sexual intercourse with your last partner?**

Yes  No



*If the respondent ticks 'Yes' on question 36 and 'No' on 57, question 58 will appear.*

**58) If you did not use a condom on that occasion, how well do the following statements agree with your situation just when you had sex? Do not take too long to think about the answers. (Tick one on each line)**

	Strongly agree	Agree	Don't know	Disagree	Strongly disagree
I trust my partner	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If I pull out a condom, my partner will think that I've been with many others before him/her	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If I pull out a condom, my partner will think I have an STI that I will not talk about	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If I pull out a condom, I've got no romantic appeal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If I pull out a condom, my partner will think we're together only for the sex	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If I pull out a condom, my partner will think that I don't want to go steady	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I don't need condoms because I know my partner well	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
We've got other contraception	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



If the respondent ticks 'Yes' on question 36, quest. 59-62 will appear

### Questions about your *last sexual intercourse*

---

**59) What was your relationship with your last sexual partner?**

- Regular partner/sweetheart
- Ex-partner/sweetheart
- A friend I have sex with
- An acquaintance
- Casual contact
- Other

**60) At last sexual intercourse, how old was your partner?  
(Use two digits, eg. 17)**

**61) How did you meet your last sexual partner, ie. on what occasion?**

- At school/work
- Through friends/family
- At a private party
- At a bar, disco, club
- On the Internet
- Other

**62) Did you use any contraception at your last intercourse?**

- None
- Yes, condoms
- Hormonal contraception (the pill, p-patch, p-injections, etc)
- IUD
- Yes, both condoms and other contraception
- Yes, emergency pill
- Other protection
- Withdrawal (coitus interruptus)
- Don't know



*If the respondent ticks 'Yes' on question 36 and 'No, Hormonal contraception, IUD, Emergency pill, Other, Withdrawal or Don't know' on 62, question 63 will appear:*

**63) Why did you not use condom at last intercourse?**

- Had no condoms available
- I was trying for a baby
- Did not worry about sexually transmitted infections
- I was unprepared
- Too drunk/ drugged up
- I would not risk losing my erection
- Dared not suggest a condom
- It feels better without
- Unsure how to put it on
- Used other contraception (the pill, IUD, etc.)
- Used other contraception (withdrawal, safe periods)
- I'm always STI tested after unprotected sex
- Other



*If the respondent ticks 'Yes' on question 36 and 'Yes, condoms' or 'Yes, condoms and other contraception', questions 64-65 will appear:*

**64) What was (were) your reasons for using a condom at last intercourse? (Please tick all reasons that apply on that occasion)**

- To avoid pregnancy
- To avoid catching a sexually transmitted infection
- To avoid HIV/AIDS
- To be more hygienic
- To avoid making a mess
- For fun
- To make sex last longer
- To make entry smoother
- Other reasons

**65) From the list above, what was your main reason for using one?**

Select answer



*If the respondent ticks 'Yes' on question 36, question 66-68 will appear:*

**66) At your last intercourse, how long had you known your partner?**

- Had met him/her for the first time on the same day/evening
- Less than a week
- 1-4 weeks
- 1-6 months
- More than 6 months

**67) Did you use alcohol or drugs in connection with last sexual intercourse?**

- No
- Yes, but I was not affected by it
- Yes, I was a little tipsy
- Yes, I was very drunk/drugged up

**68) What was the reason you had sex the last time? (Please, tick one on each line)**

- |   | Yes                      | No                       |
|---|--------------------------|--------------------------|
| Because I was in love with my partner   | <input type="checkbox"/> | <input type="checkbox"/> |
| Because my partner wanted to            | <input type="checkbox"/> | <input type="checkbox"/> |
| Beacuse my partner used pressure        | <input type="checkbox"/> | <input type="checkbox"/> |
| Curiosity/excitement                    | <input type="checkbox"/> | <input type="checkbox"/> |
| Was sexually aroused/horny              | <input type="checkbox"/> | <input type="checkbox"/> |
| Don't know, it just turned out that way | <input type="checkbox"/> | <input type="checkbox"/> |





Appendix E  
Questionnaire  
*Original Norwegian version*



# Hovedstudie: Seksualitet og klamydia i Finnmark

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**Først vil vi vite litt om din bakgrunn og hvordan du ser på deg selv**

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**1) \* Kjønn**

Gutt/mann  Jente/kvinne

**2) \* Fødselsår, fire siffer (ÅÅÅÅ):**

**3) \* Skolekommune**

Velg alternativ

**4) Hvor bor du under skolegangen?**

Hjemme hos foreldre/andre foresatte

Besteforeldre, andre slektninger

Privat hybel

'Elevhjem'

Vertsfamilie

Annet



## Utdanning

---

### 5) Hva er den høyeste utdanningen du har tenkt å ta?

- Videregående skole: Allmenn, økonomi, administrasjon, idrett eller musikk-dans-drama
- Videregående skole: Yrkesfag
- Høyskole eller universitet, 4 år eller mindre (f.eks. bachelor, lærer, politi, sykepleier, ingeniør, journalist)
- Høyskole eller universitet, mer enn 4 år (f.eks. master, lektor, advokat, sivilingeniør, lege, tannlege)
- Har ikke bestemt meg
- Annet

### 6) Hvilken utdanning har/hadde din mor?

- Ingen utdanning etter grunnskole
- Yrkesfaglig videregående skole eller tilsvarende
- Allmennfaglig videregående skole (gymnas) eller tilsvarende
- Høyskole eller universitet, 4 år eller mindre (f.eks. bachelor, lærer, politi, sykepleier, ingeniør, journalist)
- Høyskole eller universitet, mer enn 4 år (f.eks. master, lektor, advokat, sivilingeniør, lege, tannlege)
- Vet ikke

### 7) Hvilken utdanning har/hadde din far?

- Ingen utdanning etter grunnskole
- Yrkesfaglig videregående skole eller tilsvarende
- Allmennfaglig videregående skole (gymnas) eller tilsvarende
- Høyskole eller universitet, 4 år eller mindre (f.eks. bachelor, lærer, politi, sykepleier, ingeniør, journalist)
- Høyskole eller universitet, mer enn 4 år (f.eks. master, lektor, advokat, sivilingeniør, lege, tannlege)
- Vet ikke



## Kultur og kontakt

### 8) Jeg oppfatter min etnisitet som: (du kan sette flere kryss)

Norsk  Samisk  Russisk  Kvensk  Finsk  Annet

### 9) Besteforeldres (mors) etnisitet er: (du kan sette flere kryss)

Norsk  Samisk  Russisk  Kvensk  Finsk  Annet

### 10) Besteforeldres (fars) etnisitet er: (du kan sette flere kryss)

Norsk  Samisk  Russisk  Kvensk  Finsk  Annet

### 11) Har du og dine foreldre / foresatte tilhørighet til noe spesielt trossamfunn?

	Statskirke	Læstadianisme	Jehovas vitner, pinsemenighet	Russisk-ortodoks	Islam	Ingen tilhørighet
Meg selv	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Mor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Far	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



## Selvbilde

De følgende spørsmålene handler om du opplever negative tanker om deg selv. For hvert utsagn ber vi deg krysse av i boksen som du synes passer best for deg.

### 12) Å tenke negativt om meg selv er noe...

	Helt enig	Enig	Usikker	Uenig	Helt uenig
jeg gjør ofte	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
jeg gjør automatisk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
som på en måte føles naturlig for meg	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
som er typisk for meg	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
jeg har vanskelig for å la være	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
jeg begynner på før det går opp for meg at jeg gjør det	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>




## Internett

---

### 13) Hva gjør du på internett som har tilknytning til kjærlighet og seksualitet: (Du kan sette flere kryss)

- Leter etter noen å flørte med
- Leter etter kjærlighetskontakter/partnere
- Leser erotiske tekster (noveller/fortellinger)
- Ser på erotiske bilder/filmer
- Ser på pornografiske bilder/filmer
- Sjekker ut kontaktsider
- Svarer på sexannonser
- Chatter med likesinnede
- Søker seksualopplysning/rådgivning
- Kjøper sexprodukter (video, hjelpemidler, etc.)
- Kontakter prostituerte
- Jeg gjør ingenting knyttet til kjærlighet og seksualitet på internett

### 14) Har du kommet i kontakt med noen på internett som du etterpå traff og hadde sex med utenfor internett (dvs. i virkeligheten)?

- Ja
- Nei
- 

*Hvis respondenten svarer 'ja' på spørsmål 14), vil spørsmål 15-16 komme opp på skjermen.*

### 15) Hvor mange ganger har du kommet i kontakt med noen på internett som du etterpå har hatt sex med utenfor internett? (angi antallet med 2 siffer, f.eks. 02)

### 16) Hva var formålet med å treffe personen du traff og hadde sex med utenfor internett (den siste gangen det skjedde)?

- For å innlede et romantisk kjærlighetsforhold
- For å innlede et seksuelt forhold
- For å ha sex ved et enkelt tilfelle
- For å ha sex utenfor mitt faste forhold (sidesprang)

## Rus

**17) Har du noen gang prøvd, eller bruker du, noe av det følgende? (Velg ett alternativ på hver linje)**

	Aldri prøvd	Prøvd	Bruker av og til	Bruker regelmessig
Snus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sigaretter/tobakk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alkohol	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cannabis (hasj, marihuana)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Amfetamin (speed)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Ecstasy (E)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Annet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



**18) Dine følelser i forhold til seksualitet (Velg ett alternativ per linje)**

	Helt enig	Enig	Usikker	Uenig	Helt uenig
Jeg ville virkelig føle meg nervøs hvis jeg gikk inn i et seksuelt forhold med noen	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Seksuelle fantasier er sunt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Jeg liker virkelig tanken på å bli befølt seksuelt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Jeg liker virkelig tanken på å beføle noen seksuelt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**19) Har du for tiden en kjæreste?**

- Nei, jeg har aldri hatt en kjæreste
- Jeg hadde en tidligere, men ikke nå
- Ja, jeg har en nå



*Hvis respondenten svarer 'ja' på spørsmål 19, vil spørsmål 20 komme opp på skjermen.*

**20) Hvis du har en kjæreste nå, hvilket kjønn har kjæresten din?**

Gutt/mann  Jente/kvinne



*Hvis respondenten krysser 'Jeg hadde en tidligere, men ikke nå' på spørsmål 19, vil spørsmål 21 komme opp på skjermen.*

**21) Hvis du hadde en kjæreste tidligere, hvilket kjønn var kjæresten din?**

Gutt/mann  Jente/kvinne



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## Prevensjon og klamydiasmitte

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**22) Har du i løpet av det siste året gjort noe av det følgende?**

	Ja	Nei
Kjøpt kondomer	<input type="checkbox"/>	<input type="checkbox"/>
Fått kondomer gratis fra helsesøster, Ungdommens helsestasjon, organisasjon, el. annet	<input type="checkbox"/>	<input type="checkbox"/>
Øvd på å sette på kondom selv	<input type="checkbox"/>	<input type="checkbox"/>

**23) Har du i løpet av de siste 2 årene oppsøkt helsesøster på skolen, Ungdommens helsestasjon eller et legesenter for å få noe av det følgende?**

	Ja	Nei
Kondomer	<input type="checkbox"/>	<input type="checkbox"/>
Annen prevensjon	<input type="checkbox"/>	<input type="checkbox"/>
Råd/test i forbindelse med at du selv/partneren mistenkte graviditet	<input type="checkbox"/>	<input type="checkbox"/>
Råd for kjønns sykdommer (klamydia, herpes, hiv, osv.)	<input type="checkbox"/>	<input type="checkbox"/>
Test/behandling for kjønns sykdommer (klamydia, herpes, hiv, osv.)	<input type="checkbox"/>	<input type="checkbox"/>





## Reaksjon på test og risiko for klamydiasmitte

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### 24) Reaksjon på å bli klamydiatestet på skolen (ett kryss per linje)

	Helt enig	Enig	Usikker	Uenig	Helt uenig
Jeg er glad for at jeg fikk tilbud om test	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Jeg reagerte negativt over å få et slikt tilbud	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tilbud om tester påvirker meg ikke positivt eller negativt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### 25) Hvor stor risiko bedømmer du at du har for å smittes med klamydia?

- Ingen risiko
- Liten risiko
- Middels risiko
- Stor risiko
- Meget stor risiko
- Usikker/vet ikke



*Hvis respondenten krysser 'Ingen risiko' eller 'Liten risiko' på spørsmål 25, vil spørsmål 26 komme opp på skjermen.*

### 26) Årsaken til at du svarte ingen eller liten risiko er: (du kan sette flere kryss)

- Aldri har sex med noen
- Har en fast partner
- Stoler på at partneren forteller om han/hun er smittet
- Synes du klarer å vurdere på forhånd om partner er smittet eller ikke
- Alltid bruker kondom
- Annet



## Klamydiainfeksjon

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### 27) Har du tidligere blitt testet for klamydiainfeksjon?

- Ja, en gang
- Ja, 2 ganger
- Ja, 3 ganger
- Ja, 4 ganger eller flere
- Nei

### 28) Har du tidligere fått behandling for en klamydiainfeksjon?

- Ja, en gang
- Ja, 2 ganger
- Ja, 3 ganger
- Ja, 4 ganger eller flere
- Nei



*Hvis respondenten krysser 'Ja, en, 2, 3, 4 eller flere ganger' på spørsmål 28, vil spørsmål 29 komme opp på skjermen.*

### 29) Siste gang du ble behandlet for klamydia, ble din(-e) partner(-e) kontaktet for behandling?

- Ja  Nei  Vet ikke



*Hvis respondenten krysser 'Ja' på spørsmål 29, vil spørsmål 30 komme opp på skjermen.*

### 30) Siste gang du ble behandlet for klamydia, hvem tok kontakt med din(-e) partner(-e)?

- Jeg tok selv kontakt
- Helsesøster tok kontakt
- Lege tok kontakt
- Annet



**31) Har du de siste 4 uker blitt behandlet med antibiotika for klamydia eller annen infeksjon?**

Ja  Nei  Vet ikke



*Hvis respondenten krysser 'Jente' på 'Kjønn', vil spørsmål 32 komme opp:*

**32) For jenter: Har du for øyeblikket noen av følgende symptomer? (sett ett kryss på hver linje)**

	Ja	Nei
Svie ved vannlating	<input type="checkbox"/>	<input type="checkbox"/>
Endret eller økt utflod	<input type="checkbox"/>	<input type="checkbox"/>
Smerter underliv/nedre del av magen	<input type="checkbox"/>	<input type="checkbox"/>
Blødning mellom menstruasjoner	<input type="checkbox"/>	<input type="checkbox"/>
Blødning etter samleie	<input type="checkbox"/>	<input type="checkbox"/>



*Hvis respondenten krysser 'Gutt' på 'Kjønn', vil spørsmål 33 komme opp:*

**33) For gutter: Har du for øyeblikket noen av følgende symptomer? (sett ett kryss på hver linje)**

	Ja	Nei
Svie ved vannlating	<input type="checkbox"/>	<input type="checkbox"/>
Utflod fra urinrøret	<input type="checkbox"/>	<input type="checkbox"/>
Øm eller hoven pung	<input type="checkbox"/>	<input type="checkbox"/>
Utslett/kløe/sårhet på penishodet	<input type="checkbox"/>	<input type="checkbox"/>



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## Seksuell orientering

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**34) Hva regner du som din seksuelle orientering?**

- Heterofil/streit
- Lesbisk/homofil/bifil/skeiv
- Jeg er usikker på min seksuelle orientering

**35) Har du hatt sex med en person av samme kjønn som deg selv?**

Ja  Nei



**36) Har du noen gang hatt samleie?**

Ja  Nei



*Hvis respondentent krysser 'Jente' på 'Kjønn' og 'ja' på spørsmål 36, vil spørsmål 37 komme opp:*

**37) Har du sammen med en gutt noen gang:**

	Ja	Nei
Gitt han en klem, holdt rundt hverandre	<input type="checkbox"/>	<input type="checkbox"/>
Kysset	<input type="checkbox"/>	<input type="checkbox"/>
Tungekysset	<input type="checkbox"/>	<input type="checkbox"/>
Blitt befølt over hele kroppen av partneren	<input type="checkbox"/>	<input type="checkbox"/>
Befølt partneren over hele kroppen	<input type="checkbox"/>	<input type="checkbox"/>



*Hvis respondentent krysser 'Gutt' på 'Kjønn' og 'ja' på spørsmål 36, vil spørsmål 38 komme opp:*

**38) Har du sammen med en jente noen gang:**

	Ja	Nei
Gitt henne en klem, holdt rundt hverandre	<input type="checkbox"/>	<input type="checkbox"/>
Kysset	<input type="checkbox"/>	<input type="checkbox"/>
Tungekysset	<input type="checkbox"/>	<input type="checkbox"/>
Blitt befølt over hele kroppen av partneren	<input type="checkbox"/>	<input type="checkbox"/>
Befølt partneren over hele kroppen	<input type="checkbox"/>	<input type="checkbox"/>



*Hvis respondentent krysser 'Nei' på spørsmål 36, vil spørsmål 39-43 komme opp:*

**39) Har du noen gang hatt lyst til å ha samleie?**

Nei  Ja, noen ganger  Ja, ofte

**40) Har du noen gang påbegynt et samleie?**

Nei  Ja



**41) Det er mange grunner til at man ikke har hatt samleie. Marker de viktigste grunnene til at du ikke har hatt samleie enda (du kan sette flere kryss)**

- Er ikke klar for å ha samleie enda
- Er for sjenert
- Er ikke interessert i sex
- Venter på den rette personen
- Må være forelsket
- Venter til jeg gifter meg
- Har ikke hatt anledning
- Jeg mener det er galt/umoralsk
- Min partner vil/ville ikke
- Redd for å bli gravid/at partneren skal bli gravid
- Redd for at mine foreldre vil mislike det
- Ingen av vennene mine har hatt samleie enda
- Redd for at det skal gjøre vondt
- Annet

**42) Fra listen overfor, hva er hovedgrunnen til at du ikke har hatt samleie?**

Velg alternativ



**43) Vurder om påstandene som følger om seksualitet og kjønns sykdommer er riktig eller gale.**

	Riktig	Galt	Vet ikke
En kvinne kan bli gravid under samleiet uten at mannen får utløsning	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
En kvinnes sjanse for å bli gravid er mye større dersom hun får orgasme under samleie	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Menstruasjon er et tegn på kjønnsmodning hos kvinner	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Klamydia er en kjønns sykdom som både menn og kvinner kan få	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gutter utvikler ikke evnen til å få ereksjon før de kommer i puberteten	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
For å bli gravid må en kvinne ha samleie med samme mann flere ganger	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eggceller dannes i livmoren	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Onani er ufarlig	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Det å satse på "sikre perioder" er like sikkert som bruk av p-piller for å forhindre graviditet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gonore er den mest utbredte kjønns sykdommen i Norge	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



*Hvis respondenten krysser 'Ja' på spørsmål 36, vil spørsmål 44-49 komme opp:*

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**Første samleie**

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**44) Ved ditt første samleie: Hvor gammel var du? (angi alder i 2 siffer, f.eks. 17)**

**45) Ved ditt første samleie: Hvor gammel var din partner? (angi alder i 2 siffer, f.eks. 17)**

**46) Ved ditt første samleie, hvor lenge hadde du kjent din partner på forhånd?**

- Hadde møtt han/henne for første gang samme dag/kveld
- Mindre enn 1 uke
- 1-4 uker
- 1-6 måneder
- Over 6 måneder

**47) Hadde du drukket alkohol/brukt stoff i forbindelse med ditt første samleie?**

- Nei
- Ja, men var ikke beruset
- Ja, og var litt beruset
- Ja, og var svært beruset



**48) Mennesker har samleie av mange grunner. Hva var grunnen til at du hadde samleie akkurat første gangen? (sett ett kryss på hver linje)**

	Ja	Nei
Fordi jeg var forelsket/glad i partneren min	<input type="checkbox"/>	<input type="checkbox"/>
Fordi partneren ville det	<input type="checkbox"/>	<input type="checkbox"/>
Fordi alle andre hadde hatt det	<input type="checkbox"/>	<input type="checkbox"/>
Fordi jeg ble presset til det	<input type="checkbox"/>	<input type="checkbox"/>
Nysgjerrighet/spenning	<input type="checkbox"/>	<input type="checkbox"/>
Var seksuelt opphisset/kåt	<input type="checkbox"/>	<input type="checkbox"/>
For å få erfaringen	<input type="checkbox"/>	<input type="checkbox"/>
Vet ikke, bare ble slik	<input type="checkbox"/>	<input checked="" type="checkbox"/>

**49) Brukte dere noen form for beskyttelse ved ditt første samleie?**

- Nei, ingen
- Ja, kondom
- Ja, p-piller, p-stav, p-ring, p-plaster, p-sprøyte
- Ja, spiral
- Ja, både kondom og annet prevensjonsmiddel
- Ja, angrepille/dagen-derpå-pille/nødprevensjon
- Ja, annen beskyttelse
- Avbrutt samleie
- Usikker/vet ikke



*Hvis respondenten krysser 'Ja' på spørsmål 36, og 'Nei ingen', 'p-piller osv', 'Spiral', 'Angrepille', 'Annen beskyttelse', 'Avbrutt samleie', 'Usikker' på spørsmål 49, vil spørsmål 50 komme opp:*

**50) Hva var årsaken(-e) til at du ikke brukte kondom ved ditt første samleie? (du kan sette flere kryss)**

- Hadde ingen kondom for hånden
- Jeg ville ha barn
- Var ikke urolig for å smittes av kjønns sykdommer
- Var uforberedt på situasjonen
- Var påvirket av alkohol/narkotika
- Ville ikke risikere å miste ereksjonen
- Våget ikke foreslå kondom
- Det er deiligere uten
- Usikker på hvordan man setter på en kondom
- Brukte annet prevensjonsmiddel (p-piller, spiral, etc.)
- Brukte annen prevensjonsmetode (avbrutt samleie eller såkalte sikre perioder)
- Annet



*Hvis respondenten krysser 'Ja' på spørsmål 36, vil spørsmål 51 komme opp:*



**51) Har du hatt mer enn ett samleie?**

Ja  Nei

*Hvis respondenten krysser 'Ja' på spørsmål 36 og 'Ja' på spørsmål 51, vil spørsmål 52-55 komme opp:*

**52) Hvor mange samleiepartnere har du hatt i løpet av de siste 6 månedene? (angi hele tall i to siffer, f.eks. 02)**

**53) Hvor mange samleiepartnere har du hatt i løpet av de siste 12 månedene? (angi hele tall i to siffer, f.eks. 02)**

**54) Hvor mange samleiepartnere har du hatt totalt? (angi hele tall i to siffer, f.eks. 02)**



**55) Hvor lenge er det siden siste gang du hadde samleie?**

- Mindre enn 1 uke
- 1-4 uker
- 1 måned-mindre enn 3 måneder
- 3 måneder til 1 år
- Over 1 år siden



*Hvis respondenten krysser 'Ja' på spørsmål 36 og 'Mindre enn 1 uke' eller '1-4 uker' på spørsmål 55, vil spørsmål 56 komme opp:*

**56) Omtrent hvor mange samleier har du hatt den siste måneden?**

- 1 samleie
- 2-5 samleier
- 6-9 samleier
- 10-30 samleier
- Over 30 samleier



*Hvis respondenten krysser 'Ja' på spørsmål 36, vil spørsmål 57 komme opp:*

## Spørsmål om den første gangen du hadde samleie med din siste sex-partner

**57) Ble det brukt kondom første gang du hadde samleie med din siste partner?**

Ja  Nei



*Hvis respondenten krysser 'Ja' på spørsmål 36 og 'Nei' på 57, vil spørsmål 58 komme opp:*

**58) Hvis du ikke brukte kondom ved denne anledningen, hvor godt stemmer påstandene nedenfor med din situasjon akkurat da du hadde samleie**

*Ikke bruk for lang tid til å tenke gjennom svarene (Sett ett kryss per linje)*

	Stemmer svært godt	Stemmer ganske godt	Usikker	Stemmer ganske dårlig	Stemmer svært dårlig
Jeg stoler på partneren	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Om jeg drar opp et kondom, vil partneren tro jeg har vært sammen med mange før han/henne	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Om jeg drar opp et kondom, vil partneren tro jeg har en kjønns sykdom som jeg ikke vil fortelle om	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Om jeg drar opp et kondom, vil det bli uromantisk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Om jeg drar opp ett kondom, vil partneren tro jeg bare er ute etter sex	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Om jeg drar opp et kondom, vil partneren tro jeg ikke er ute etter å bli sammen med han/henne	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Kondom er unødvendig fordi jeg kjenner partneren godt fra før	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Vi har annen prevensjon	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



Hvis respondentent krysser 'Ja' på spørsmål 36, vil spørsmål 59-62 komme opp:

### Spørsmål om siste gang du hadde samleie

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**59) Hvilket forhold har du til den du hadde samleie med siste gang?**

- Fast partner/kjæreste
- Tidligere partner/kjæreste
- Venn ("knullevenn/knullevenninne")
- Bekjent
- Tilfeldig kontakt/en jeg ikke kjente på forhånd
- Annen

**60) Ved ditt siste samleie: Hvor gammel var din partner? (angi alder i 2 siffer, f.eks. 17)**

**61) Hvor traff du din siste partner? Ved hvilken anledning?**

- Gjennom skole/arbeid
- Gjennom venner/familie
- På privatfest
- På et utested (diskotek, kafe, bar, osv.)
- På Internett
- Annet

**62) Brukte dere noen form for beskyttelse ved ditt siste samleie?**

- Nei, ingen
- Ja, kondom
- Ja, p-piller, p-stav, p-ring, p-plaster, p-sprøyte
- Ja, spiral
- Ja, både kondom og annet prevensjonsmiddel
- Ja, angrepille/dagen-derpå-pille/nødprevensjon
- Ja, annen beskyttelse
- Avbrutt samleie
- Usikker/vet ikke

*Hvis respondenten krysser 'Ja' på spørsmål 36, og på spørsmål 62: 'Ingen, p-piller osv, Spiral, Angrepille, Annen, Avbrutt eller Vet ikke', vil spørsmål 63 komme opp:*

**63) Hvorfor brukte du ikke kondom ved ditt siste samleie? (du kan sette flere kryss)**

- Hadde ingen kondom for hånden
- Jeg ville ha barn/var gravid
- Var ikke urolig for å smittes av kjønns sykdom
- Var uforberedt på situasjonen
- Var påvirket av alkohol/narkotika
- Ville ikke risikere å miste ereksjonen
- Våget ikke foreslå kondom
- Det er deiligere uten
- Usikker på hvordan man setter på et kondom
- Brukte annet prevensjonsmiddel (p-piller, spiral, etc.)
- Brukte annen prevensjonsmetode (avbrutt samleie eller såkalte sikre perioder)
- Tar alltid en test for kjønns sykdom etter ubeskyttet sex
- Annet



*Hvis respondenten krysser 'Ja' på spørsmål 36, og 'Kondom' eller 'Kondom og annen prevensjon' på spørsmål 62, vil spørsmål 64-65 komme opp:*

**64) Hvilke grunner hadde du for å bruke kondom ved ditt siste samleie? (du kan sette flere kryss)**

- For å unngå graviditet
- For å unngå smitte med kjønns sykdom
- For å unngå smitte med HIV/AIDS
- For å være mer renslig
- For å unngå å søle
- For moro skyld
- For å få sexen til å vare lenger/ikke komme så fort
- For å gjøre inntrenging lettere
- Annet

**65) Fra listen overfor, hva var hovedgrunnen til kondombruken?**

Velg alternativ



*Hvis respondenten krysser 'Ja' på spørsmål 36, vil spørsmål 66-68 komme opp:*

**66) Ved ditt siste samleie, hvor lenge hadde du kjent din partner på forhånd?**

- Hadde møtt han/henne for første gang samme dag/kveld
- Mindre enn 1 uke
- 1-4 uker
- 1-6 måneder
- Over 6 måneder

**67) Hadde du drukket alkohol/brukt stoff i forbindelse med ditt siste samleie?**

- Nei
- Ja, men var ikke beruset
- Ja, og var litt beruset
- Ja, og var svært beruset

**68) Hva var grunnen til at du hadde samleie akkurat siste gangen? (Sett ett kryss på hver linje)**

- |  | Ja                       | Nei                      |
|--|--------------------------|--------------------------|
| Fordi jeg var forelsket/glad i partneren min | <input type="checkbox"/> | <input type="checkbox"/> |
| Fordi partneren ville det                    | <input type="checkbox"/> | <input type="checkbox"/> |
| Fordi jeg ble presset til det                | <input type="checkbox"/> | <input type="checkbox"/> |
| Nysgjerrighet/spenning                       | <input type="checkbox"/> | <input type="checkbox"/> |
| Var seksuelt opphisset                       | <input type="checkbox"/> | <input type="checkbox"/> |
| Vet ikke, ble bare slik                      | <input type="checkbox"/> | <input type="checkbox"/> |



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