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Investigation of an imported case of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in Florence, Italy, May to June 2013

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On 31 May 2013, the first case of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in Italy was laboratory confirmed in a previously healthy adult man, who developed pneumonia with moderate respiratory distress after returning from a holiday in Jordan. Two secondary cases were identified through contact tracing, among family members and colleagues who had not previously travelled abroad. Both secondary cases developed mild illness. All three patients recovered fully.

On 31 May 2013, the National Influenza Centre at the Istituto Superiore Sanità (NIC-ISS) in Rome, Italy, confirmed the first case of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in a patient, hospitalised in Florence (Tuscany region), Italy, who had just spent a vacation in Amman, Jordan. Two secondary cases of MERS-CoV were subsequently detected among close contacts. We here report the public health investigation carried out and the identification and follow-up of the three patients' contacts.

Index case

The index case was a previously healthy man in his mid-40s who had returned to Florence on 25 May 2013, following a 40-day holiday in Jordan (Figure). The patient developed influenza-like symptoms the previous day, while in Jordan, and was symptomatic on the flight back to Italy and while back at work in a hotel on 27 May. On 28 May, because of worsening symptoms, he visited the emergency room of a local hospital (Hospital A) in Florence and was admitted later the same day to the Infectious and Tropical Diseases Unit of a second hospital (Hospital B), in the same city. His symptoms at that time were fever (38 °C), cough and difficult breathing. A chest radiograph revealed signs of pneumonia, with bilateral lung infiltrates. The following day, testing for MERS-CoV was carried out (real-time polymerase chain reaction (PCR) to detect the regions upstream of the E gene (upE region)) on upper respiratory samples (nasopharyngeal swabs) at the Regional reference laboratory for Influenza at the University of Florence and MERS-CoV infection was diagnosed. This result was confirmed by the National Influenza Centre [1].

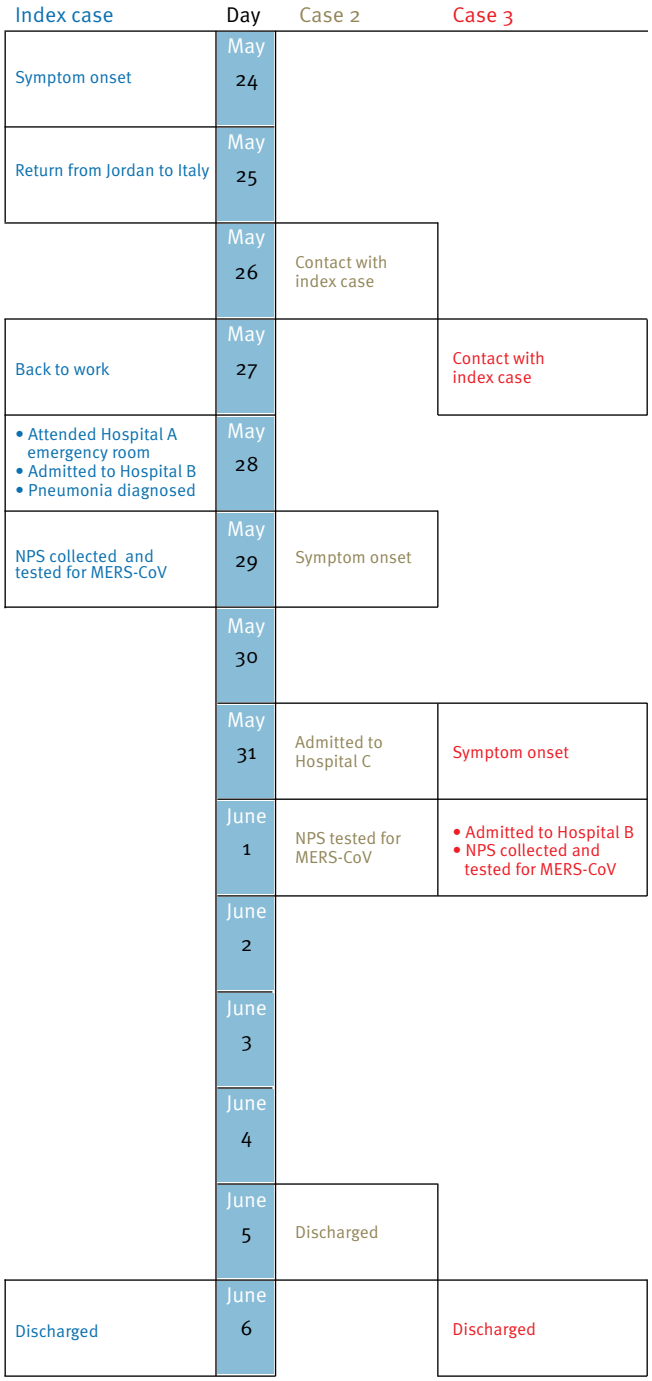
On 29 May, as soon as the clinicians began to suspect a MERS-CoV infection, the staff of Hospital B complied with all infection control procedures, according to the Health Protection Agency (HPA) (now Public Health England) infection control advice for suspected or confirmed novel coronavirus cases [2]. In particular, the patient was isolated in a negative-pressure room and staff wore protective clothing and performed hand hygiene. Attempts were made at the Regional reference laboratory for Influenza in Florence to isolate the virus in Vero cells, but were unsuccessful.

Identification of close contacts

According to a standard definition of 'close contact' [3], 115 contacts of this patient were identified and placed under surveillance: 90 in the healthcare setting (14 healthcare workers (HCWs) in Hospital A, two HCWs of the local health district (ASL 10) of Florence, 28 HCWs of Hospital B, one patient who shared the same hospital room (before the index case was placed in isolation), five cleaners of Hospital B, who worked in the room of the index case (before he was placed in isolation) and three ambulance operators); in addition, the surveillance included 37 patients who attended the emergency room in Hospital A at the same time of the index case); four family members; nine from the aeroplane

Figure

Timeline of three cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection, Italy, 24 May–6 June 2013



NPS: nasopharyngeal swab.

(the names of the passengers who were on the same return flight of the index case, with an assigned seat in the same row and in the two rows in front and behind him, were easily obtained but the exact position of these contacts on the plane was difficult to assess as some passengers could have changed their assigned seats); and 12 at the patient's place of work.) However, it must be underlined that, although particular attention was given to people who had had prolonged face-to-face contact with the index case (duration of at least 15 minutes within one metre from the confirmed case), some of the above 115 individuals placed under clinical surveillance, mainly from the healthcare setting, may have had a lesser degree of contact with him or were already wearing personal protective equipment at the time of contact.

Cases 2 and 3

On 29 May, the index case's relation (Case 2) aged about a year and a half developed a mild febrile illness (Figure). She had been in close contact with the index case on May 26, when the man spent all the day with her and her family, staying in the same room. As she was under surveillance, she was admitted to a children's hospital (Hospital C) in Florence, on 31 May and a nasopharyngeal swab was taken. This tested positive for MERS-CoV, by real-time PCR for the upE region, the following day. Five family members of the child underwent clinical surveillance.

On 31 May, a female co-worker of the index case (Case 3), in her early 40s, who shared the same office with the index case on 27 May, developed influenza-like illness (fever (37.5 °C)and cough) (Figure). She was admitted to the Infectious Diseases Unit of Hospital B in Florence on 1 June and a nasopharyngeal swab tested positive for MERS-CoV, by real-time PCR for the upE region, the same day. Her five family members (husband, three sons and her father) were also placed under clinical surveillance. A further three close contacts (two friends and a family general practitioner) were identified: these eight contacts plus 16 work colleagues were monitored clinically, bringing the total number of Case 3's contacts placed under surveillance to 24.

As for the index case, the nasopharyngeal swabs of Cases 2 and 3 were tested for MERS-CoV by real time PCR for the upE gene [1]. According to guidance documents of the World Health Organization (WHO) [4], these laboratory results were interpreted as 'presumptive' evidence of MERS-CoV infection in both cases. The final classification as confirmed cases was given on the basis of laboratory data combined with clinical/epidemiological information available.

Case definition and clinical surveillance of contacts

In Italy, a possible case of MERS-CoV infection is defined as follows:

- any patient with an acute respiratory infection, which may include history of fever (≥38 °C) and cough and indications of pulmonary parenchymal disease (e.g. pneumonia or acute respiratory distress syndrome (ARDS)), AND
- whose illness cannot be explained by the presence of other infections, AND
- who had a history of travel to or residence in affected areas (in the Middle East), during the 10 days before symptom onset, OR
- having had close contact, during the 10 days before symptom onset, with a symptomatic confirmed case of MERS-CoV infection.

On the basis of WHO and ECDC guidance documents [5,6], a probable case of MERS-CoV infection is defined as follows:

- a person with a febrile acute respiratory illness with clinical, radiological or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or ARDS), AND
- for whom MERS-CoV infection has not been laboratory confirmed, AND
- who has no direct epidemiological link to a confirmed MERS-CoV case.

A confirmed case is defined as a person with laboratory confirmation of MERS-CoV infection [5,6].

According to national guidelines [7], for each possible case, clinical samples from the upper respiratory tract (nasopharyngeal swabs), as well as lower respiratory specimens (sputum or bronchoalveolar lavage fluid, when possible), have to be collected and tested for MERS-CoV in an initial screen by the regional reference laboratory; positive results have to be confirmed by the National Influenza Centre at ISS.

As soon as the index case was suspected to be infected with MERS-CoV, exposed individuals were identified through contact tracing and a clinical surveillance protocol was followed. All contacts were followed up during a 10-day period (i.e. the maximum incubation period, according to the knowledge of the disease at the time of the investigation described in this report) [5] after their last contact with the index case, to check if symptoms appeared. Particular attention was given to people who had had prolonged face-to-face contact with the index case (duration of at least 15 minutes within one metre from the confirmed case).

Discussion

As of 1 August 2013, 94 laboratory-confirmed cases of MERS-CoV infection have been reported worldwide since the first detection of this novel virus in Saudi Arabia in 2012, including 46 deaths [8].

In this report, we present the investigation of the first case of MERS-CoV diagnosed in Italy and also present evidence of limited person-to-person transmission of MERS-CoV, to two people who had close contact with the symptomatic index case when he was back in Italy and who had not previously travelled abroad. Both secondary cases were classified as confirmed on the basis of a combination of clinical (influenza-like illness), epidemiological (close contact with the index case) and laboratory data, according to WHO guidelines [4]. Nasopharyngeal swabs of both secondary cases tested positive only in the screening assay (real-time PCR to detect the regions upstream of the E gene), probably due to the known limitations when using upper respiratory tract specimens [5,9]. Although required [7], lower respiratory tract specimens were not available.

It must be underlined that the index case was a previously healthy middle-age man, who developed fever and respiratory symptoms on 24 May, i.e. the day before his travel from Jordan to Italy. For the two secondary cases, the putative incubation time (i.e. days from putative exposure to symptom onset) was 3-4 days, according to data already reported [10,11]. The incubation period was somewhat shorter than that found for the Severe Acute Respiratory Syndrome (SARS)-coronavirus infection [12].

All three patients, including the index case who developed pneumonia, fully recovered in less than two weeks after symptom onset, unlike most of the MERS-CoV severe cases reported [10,11]. Nevertheless, it should be stressed that none of the three patients had underlying clinical conditions. In this regard, it is also important to highlight recent findings [10] that suggested the disease is milder in people who were identified through contact tracing, compared with that seen in those presenting with symptoms.

None of the other 144 contacts monitored (115 for the index case, 5 for Case 2 and 24 for Case 3) developed fever or other symptoms suggestive of an acute respiratory illness after 10 days' follow-up. Respiratory specimens have been collected from 70 of the contacts and have tested negative for MERS-CoV.

As useful additional information can be obtained from serological investigations, we will analyse sera collected from the three cases. Furthermore, although rapid contact tracing was undertaken, identification and follow-up of a larger number of contacts and the collection of serum samples would be of great value, to better determine the potential presence of subclinical infections. Notably, an increasing proportion of people with asymptomatic infection have been recently reported [9,13], through contact tracing among close contacts of cases.

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Conflict of interest

None declared.

Authors' contributions

Simona Puzelli: coordinated the laboratory investigation for case confirmation. Simona Puzelli, Maria Rita Castrucci and Isabella Donatelli: wrote the manuscript. Alberta Azzi: supervised and coordinated the initial laboratory investigation on the three cases and contacts and was responsible for testing and interpretation of results from respiratory samples. Maria Grazia Santini and Simonetta Baretti: coordinated the contact surveillance. Angela Di Martino, Marzia Facchini and Monica Meola: performed laboratory testing for case confirmation. Rosaria Arvia, Fabiana Corcioli and Federica Pierucci: performed the initial laboratory testing on patients and contacts. Alessandro Bartoloni: provided data on the index case. Dario Bartolozzi: provided data on the co-worker of the index case (Case 3). Maurizio de Martino and Luisa Galli: cared for the child (Case 2). Giovanni Rezza, Isabella Donatelli and Maria Grazia Pompa: supervised the investigation and coordinated the relationships with health authority at national and international level. Emanuela Balocchini: supervised and coordinated the investigation at regional level. All co-authors: provided comments and revised the manuscript.

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Acute hepatitis E complicated by Guillain-Barré syndrome in Portugal, December 2012 – a case report

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Autochthonous hepatitis E virus (HEV) infection has been increasingly reported in Europe and the United States, mostly arising from genotype 3 and less frequently genotype 4. We report here on a patient with HEV genotype 3a infection complicated by Guillain-Barré syndrome in Portugal in December 2012. We draw attention to the diagnosis of autochthonous HEV infection and to its rare, but important, neurological complications.

Case report

We report on a patient in Portugal with autochthonous acute hepatitis E complicated by Guillain–Barré syndrome (GBS).

Clinical description

At the end of November 2012, a man in his late 50s was hospitalised, having presented with signs and symptoms of acute hepatitis. He had not travelled outside Portugal and had no history of risky sexual behaviour or drug addiction. Two weeks before hospitalisation, he complained of malaise, nausea, vomiting and right upper quadrant abdominal pain: these symptoms were followed by onset of jaundice some days after. At admission (day 1), besides jaundice, he had right upper quadrant tenderness and an enlarged liver.

During the three days after admission, he began to complain of muscle weakness and was then admitted to an intermediate care unit (three days after admission). After being observed by a neurologist on day 4, the patient was treated with intravenous immune globulin (0.4g per kg of body weight per day for five days) and ceftriaxone (4g/day for 10 days). During that day and the following day, a rapid ascending weakness and sensitivity loss with a non-impaired mental status was observed. At this point, the presumed diagnosis was GBS. This worsening of neurological symptoms led to the patient being transferred to our hospital on day 5, where he was admitted to the intensive-care unit (ICU). Due to hypoventilation, decreased reflex cough and hoarseness, the patient needed intubation, ventilation support and sedation. Besides the tetraparesis and areflexia, the patient also had autonomic instability with bradycardia and hypertension.

The day before admission to the ICU, magnetic resonance imaging of the spinal cord was normal. Electromyography nerve conduction studies of peripheral motor and sensory nerves in upper and lower limbs the day following ICU admission revealed a severe acquired demyelinating sensory and motor polyneuropathy.

A tracheostomy was performed five days after ICU admission and sedation was stopped. The patient began a rehabilitation programme; he was fully able to breathe spontaneously at day 26. His clinical evolution was favourable: he had progressive recovery of muscle strength, with efficacious cough and no need for oxygen supplementation. He was discharged 34 days after admission to the ICU. Two months later, he continued to have a favourable outcome and was already able to walk if assisted.

Laboratory analysis

The results of blood tests taken at various time points are shown in Table 1.

Analysis of the patient's cerebrospinal fluid (CSF) taken on day 2 after ICU admission showed normal cell count (4 cells/µL; norm: ‹5/µL) and level of proteins (181 mg/dL; norm: 15–45 mg/dL) and glucose (77 mg/dL; norm: ›60 mg/dL). The CSF and blood cultures were negative for bacteria.

Serum samples (taken on admission to the referring hospital and on admission to the ICU) and CSF (taken

Table 1

Blood test results, case of acute hepatitis E, Portugal, November–December 2012

Item tested (units)	Laboratory results			Reference values
	Admission to referring hospital	Transfer to ICU	ICU discharge (34 days after admission)	
Haemoglobin (g/dL)	14.3	12.8	11.4	13–18
Platelets (x 109/L)	328	308	286	150–400
AST (U/L)	–	79	72	‹37
ALT (U/L)	2,320	101	156	‹37
G-GT (U/L)	566	367	69	‹49
AF (U/L)	336	274	155	‹155
Total bilirubin (mg/dL)	6.71	1.13	0.4	‹1.2
Conjugated bilirubin (mg/dL)	5.5	0.8	0.18	‹0.4
Albumin (g/L)	–	27.9	–	38–51
CRP (mg/L)	60.5	50.3	8.1	‹3
aPTT (seconds)	41	35.1	–	–
PT (seconds)	12.4	12.3	–	–

AF: alkaline phosphatase; ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; CRP: C-reactive protein; G-GT: gamma-glutamyl transpeptidase; ICU: intensive-care unit; PT: prothrombin time; U: units.

The dashes indicate that the test was not done.

on admission to the ICU) were tested by serology and polymerase chain reaction to detect the presence of various pathogens (Table 2). Although *Toxoplasma* and *Mycoplasma* infections were initially suspected, based on the presence of specific IgM in the patient's serum, polymerase chain reaction (PCR) of CSF and blood samples did not confirm the presence of these agents.

Serological analysis to detect hepatitis viruses was negative for all except HEV, being positive for anti-HEV IgM and negative for anti-HEV IgG, suggesting an acute infection with HEV. Serological markers for Epstein–Barr vírus (EBV) – EBV nuclear antigen and viral capsid antigen IgG – were positive, but EBV IgM was negative. The diagnosis of HEV infection was confirmed by the presence of HEV RNA in the serum sample taken the day the patient was admitted to the referring hospital. CSF and stool samples were not available at that time for testing. HEV RNA in serum was detected using a nested broad-spectrum reverse transcription PCR with amplification within the open reading frame (ORF) 1 region of HEV genome [1].

The amplicon obtained (330 bp) was sequenced and compared with reference HEV strains, using a neighbor-joining method based on the Jukes-Cantor model [1] for ORF 1 nucleotide sequences of selected HEV isolates representing genotypes 1–4. This phylogenetic analysis revealed that the amplicon clustered with HEV genotype 3, specifically subgenotype 3a (Figure).

In a routine visit to the hospital two months after being discharged, analysis of a blood sample collected at that time showed that HEV RNA was undetectable, anti-HEV IgM was negative and anti-HEV IgG was positive.

Background

In recent years, an increasing number of autochthonous infections with HEV have been reported in Europe and in United States [2]. Most of these autochthonous cases have been caused by HEV genotype 3 (HEV3) and less frequently by genotype 4 [2]. Seroprevalence studies also show that HEV infection in the population of industrialised countries is higher than previously thought [3]. HEV3 infections have been associated with the consumption of raw or insufficiently cooked pork, deer and wild boar [2,4] and to direct contact with infected swine [5].

Autochthonous HEV infection is usually subclinical or runs a mild course and is self-limiting, but chronic autochthonous infection has been identified among immunocompromised persons, including organ transplant recipients, patients receiving cancer chemotherapy and persons infected with human immunodeficiency virus (HIV) [6].

A broad set of symptoms of autochthonous HEV disease has been seen as well as increasing recognition of its extra-hepatic manifestations [7]. Both acute and chronic HEV infections have been reported to be associated with muscular weakness, neurological disorders including polyradiculopathy, GBS, bilateral brachial neuritis, encephalitis or proximal myopathy [8]. Although neurological disorders associated with HEV3 are rare, some data are emerging in literature [8]. In

Table 2

Serological and PCR analysis to detect various pathogens, case of acute hepatitis E, Portugal, December 2012

Pathogen	Serology results	PCR results	
		Serum[a]	CSF[b]
HEV	IgM positive	Positive	–
HAV	Negative	–	–
HBV	Negative	–	–
HCV	Negative	–	–
HIV	Negative	–	–
HSV-1	IgG positive	–	–
HSV-2	Negative	–	–
CMV	Negative	Negative	Negative
EBV	IgG positive, IgM negative	–	Negative
Toxoplasma	IgM positive	Negative	Negative
Mycoplasma	IgG positive IgM positive	–	Negative
Bartonella	–	–	Negative
Borrelia	Negative	Negative	Negative
Leptospira	–	–	Negative
Coxiella	Negative	Negative	–

CMV: cytomegalovirus; CSF: cerebrospinal fluid; EBV, Epstein–Barr vírus; HAV, hepatitis A virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HEV: hepatitis E virus; HIV: human immunodeficiency virus; HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; ICU: intensive-care unit; PCR, polymerase chain reaction.

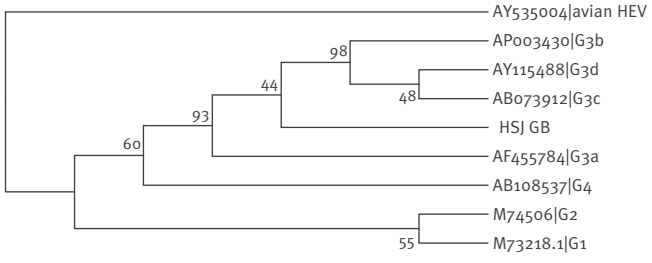
The dashes indicate that the test was not done.

[a] Taken on admission to the referring hospital and on admission to the ICU.

[b] Taken on admission to the ICU.

Figure

Phylogenetic analysis of a hepatitis E virus amplicon from a case of acute hepatitis E, Portugal, December 2012



HEV: hepatitis E virus.

Neighbor-joining method based on the Jukes-Cantor model for open reading frame (ORF) 1 nucleotide sequences of selected HEV isolates representing genotypes 1–4. An avian HEV sequence was used as an outlier. The sequence of the case (HSJ GB) is shown in bold. Sequences are denoted by the GenBank acession number followed by genotype (G) and subgenotype for G3. Numbers at the nodes indicate bootstrap values. The tree was constructed by using a 334 base pair (bp) region. Evolutionary analyses were conducted in MEGA5.

recent years, a number of GBS cases associated with HEV infections have been described [8,9]. GBS is an acute, acquired, autoimmune disorder of peripheral nerves that develops in susceptible individuals after infection and, in rare cases, after vaccination [10]. In about 60% of cases, GBS is preceded by an infection, most frequently by *Campylobacter jejuni*, but other pathogens, such as viruses from Herpesviridae family (cytomegalovirus, varicella zoster virus, Epstein–Barr virus) or bacterial agents (*Haemophilus influenzae, Mycoplasma pneumoniae*) can be responsible [10].

Discussion

Exhaustive tests to detect the agents mostly frequently associated with GBS, other than *Campylobacter*, gave negative results in samples from our patient. *Campylobacter* was not tested for because of the patient's symptoms. Although rare, the increasing number of reports of neurological disorders associated with autochthonous HEV infections in Europe drew our attention to a possible HEV diagnosis. Given the serological results, EBV was not considered likely as the causative agent. Detection of both IgM anti-HEV and HEV RNA in the patient's serum confirmed acute hepatitis E.

The actual incidence of GBS associated with HEV infection is unknown because autochthonous hepatitis E is still underdiagnosed in many industrialised countries [11]. This is in part due to the fact that frequently HEV infection is subclinical and because the neurological findings surpass the liver injuries and hepatitis is not suspected. Hence, HEV infection should be considered in neurological diseases associated with abnormal levels of liver enzymes [7,12].

Since the patient reported no travel history, it seems likely that the HEV infection was locally acquired. We consider it most probably resulted from consumption of pork or pork products, as there is a strong tradition of pork consumption among Portuguese people and HEV3 has been detected in domestic pigs from several farms in Portugal [13].

Interestingly, the patient was in his late 50s. It is known that middle-aged and elderly men are more vulnerable to severe HEV3 infection, which can ultimately lead to hospitalisation and possibly death [6].

To our knowledge, this is the first report of a neurological disorder associated with an autochthonous HEV3 infection in a Portuguese patient. Considering the GBS-HEV cases reported, we recommend that testing for HEV should be included routinely in the diagnostic algorithm of GBS when liver function is altered.

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Conflict of interest

None declared.

Authors' contributions

LS, NRP took part in the clinical management of the patient and wrote the manuscript. CLA, RS, PF, JR, AS took part in the clinical management of the patient and reviewed the manuscript. JS collaborated in molecular biology techniques. MSJN and JRM did the molecular diagnosis tests and the sequence of the HEV and wrote the lab section. All authors read and approved the final manuscript.

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Lymphogranuloma venereum among men who have sex with men in the Netherlands: regional differences in testing rates lead to underestimation of the incidence, 2006-2012

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Since 2003, an epidemic of lymphogranuloma venereum (LGV) has been ongoing in men who have sex with men (MSM) in Europe. Of 92,271 MSM consulting sexually transmitted disease (STI) clinics in the Netherlands between 2006 and 2011, 63,228 (68%) were tested for anorectal *Chlamydia* infection, with 6,343 (10%) positive diagnoses. In 4,776 of those (75%), LGV testing was performed, with regional variation from 7% to 97%. In total 414 LGV cases were diagnosed, a mean annual positivity rate of 8.7%, decreasing from 14% in 2007 to 6% in 2011, but increasing to 13.1% during 2012 (184 new cases). Risk factors for LGV were human immunodeficiency virus (HIV) positivity (odds ratio (OR)=4.1; 95% confidence interval (CI): 3.2–5.3), STI symptoms (OR=4.1; 95% CI: 3.1–5.4), more than 50 sex partners in the past six months (OR=3.7; 95% CI: 1.1–12.4), older age (40–44 years: OR=2.1; 95% CI: 1.5–2.8), no condom use (OR=2.2; 95% CI: 1.2–3.9) and homosexuality (as opposed to bisexuality; OR=2.2; 95% CI: 1.1–4.2). Regional differences in LGV testing rates limit national LGV surveillance, leading to an underestimation of the real incidence. Characteristics of MSM with LGV did not change over time, so existing prevention strategies should be intensified.

Introduction

Men who have sex with men (MSM) are generally considered to be at increased risk for a range of sexually transmitted infections (STIs), including gonorrhoea and human immunodeficiency virus (HIV) infection [1]. This is supported by the observation that in the Netherlands in 2010, 19% of MSM attending an STI clinic were diagnosed with one or more STIs, compared with 12% of heterosexual attendees [1].

Since 2003, an epidemic of lymphogranuloma venereum (LGV) has been ongoing among MSM in Europe [2]. The first cases of the epidemic were reported in Rotterdam, the Netherlands [3,4]. Before 2003, the incidence of LGV in the Netherlands had been approximately five cases annually, and LGV was considered to be a rare tropical disease endemic in other areas of the world, including Africa, India, Asia and the Caribbean [5]. The 1,693 cases reported during this epidemic showed several similarities: they were all male, specifically MSM, over 25 years-old, and the majority (80–100%) was HIV-positive [6]. After careful investigation of this first Dutch outbreak, sexual contacts of these patients were traced to Belgium, France, Germany and the United Kingdom [4], which could explain the rapid spread of the disease throughout Europe.

LGV is a bacterial infection caused by the L1, L2 or L3 serovars of the intracellular bacterium *Chlamydia trachomatis* [7]. *C. trachomatis* infection with serovars D to K is mostly associated with mild to asymptomatic infection. However, LGV is considered to be a more invasive infection that results in symptomatic infection in the majority of cases [8]. The ulcerative nature of LGV has been suggested to be associated with increased STI transmission, for example of HIV and other blood-borne infections such as hepatitis C [3,7].

After the first cluster of LGV cases was reported in January 2003, enhanced surveillance of the disease was implemented in the Netherlands [9]. Increased awareness of the disease resulted in the development of a European guideline advising on control measures [10]. The two main recommended interventions were partner

notification and monitoring patients until all symptoms have disappeared, to prevent further spread.

The primary guideline for LGV testing at STI clinics in the Netherlands was developed by the National Preparedness and Response Unit of the Dutch Centre for Infectious Disease Control (CIb/LCI) at the National Institute for Public Health and the Environment (RIVM) [11]. This guideline recommends LGV testing for all MSM presenting with clinical symptoms. Furthermore, the Dutch Society for Dermatology and Venereology (NVDV) published in 2005 their recommendation that LGV testing should be performed for all MSM with anorectal chlamydia [12]. The guideline developed by the NVDV is a broader second-line guideline, which may also be considered by STI clinics in addition to the RIVM guidelines. However, it is currently being discussed whether only HIV-positive MSM and MSM showing STI symptoms should be tested for LGV.

This study aims to provide an update on how the epidemic of LGV among MSM in the Netherlands developed over the period from January 2006 to December 2012, and its implications for current and future control policy, by analysing quantitative STI surveillance data.

Methods

Study sample and design

This study is a time-trend analysis of surveillance data, including data on MSM attending any STI clinic in the Netherlands between 1 January 2006 and 31 December 2011. MSM were defined as men who have sexual contacts either exclusively with men or with both men and women. The dataset contained 92,271 consultations by MSM in this time period, and data collection did not allow for identification of repeat visits of the same individual. In addition, preliminary data on LGV testing and diagnoses of the first half of 2012 were included.

Data collection

At the STI clinics, STI consultations were conducted anonymously and reported to the RIVM facilitated by a web-based application called SOAP. For all clients, information on demographics and behavioural risks was collected by an interview with a nurse or medical doctor. Each STI consultation involved laboratory testing and medical examination. Clients were routinely tested for chlamydia, gonorrhoea, syphilis and HIV infection [1].

LGV diagnosis is a two-step process. First, samples obtained from clinic attendees were routinely tested for chlamydia. According to the Dutch guidelines, anorectal testing for chlamydia was performed based on reported risk behaviour and/or symptoms [11,12]. If the anorectal chlamydia test is positive, further testing for LGV serovars can be performed.

Data analysis

Characteristics of MSM were investigated by applying descriptive statistics. Within the group of MSM tested for LGV, chi-square testing was applied to investigate whether there was a significant association ($p<0.05$) between the characteristics and LGV test result.

Based on the dates of the consultations, time trends for the positivity rate for LGV were investigated. Positivity rates were obtained by calculating the proportion of LGV-positive cases among all MSM in whom LGV testing was performed. Furthermore, the proportion of anorectal chlamydia-positive MSM for whom LGV testing was performed was calculated to investigate whether there were geographical differences in testing practice. We followed the division of the Netherlands into eight different regions with one central STI clinic per region, as described in Vriend et al. [1].

Univariate logistic regression analysis was performed to select relevant variables to be included in multivariate logistic regression analysis for risk factors for LGV infection. Variables with a significance of $p<0.20$ were included in subsequent analyses. The effect of the variables was investigated for LGV-positive MSM compared with LGV-negative MSM. LGV-negative MSM were defined as MSM with an anorectal *Chlamydia* infection, but with a negative LGV test result. MSM with a negative anorectal chlamydia test were defined as anorectal chlamydia-negative MSM. MSM without an anorectal chlamydia test were defined as not anorectally tested MSM.

A backward stepwise approach was selected to investigate which variables were significant risk factors for LGV infection. For multivariate analysis, the confidence level for statistical significance was set at $p\leq0.05$. To investigate time trends, these analyses were also performed after stratification by year. All statistical analyses were carried out using IBM SPSS Statistics 19.

In addition, a crude estimation was made to investigate how many cases could potentially be missed among the anorectal chlamydia-positive MSM for whom LGV testing was not performed. This estimation was made by extrapolating positivity rates for significant risk factors to the population of anorectal chlamydia-positive MSM for whom LGV testing was not performed. Differences in diagnostics and number of LGV patients between STI clinics were not taken into account in this estimation.

Results

Study population

In 63,228 of the 92,271 (69%) consultations of MSM between 2006 and 2011, an anorectal *Chlamydia* infection was tested for, with 6,343 positive anorectal chlamydia diagnoses, a positivity rate of 10%. In 4,776 of these 6,343 consultations (75%), LGV testing was performed and between 2006 and 2011, 414 cases of LGV were diagnosed (Table 1).

Table 1

Characteristics of MSM with an anorectal *Chlamydia* infection, MSM tested for LGV, MSM diagnosed with LGV and LGV positivity rate of MSM visiting an STI clinic in the Netherlands, 2006–2011 (n=6,343)

		Anorectal CT-positive MSM	MSM tested for LGV	LGV-positive MSM		p value
		Number	Number	Number	% positive	
Total		**6,343**	**4,776**	**414**	**8.7**	
Year of consultation	2006	572	342	34	9.9	<0.001
	2007	710	464	65	14.0	
	2008	1,061	772	98	12.7	
	2009	1,082	867	82	9.5	
	2010	1,381	1,136	66	5.8	
	2011	1,537	1,195	69	5.8	
Age group[a]	<35	2,788	2,008	97	4.8	<0.001
	35–39	998	806	81	10.0	
	40–44	991	774	109	14.1	
	>45	1,565	1,188	127	10.7	
Origin[b]	The Netherlands	4,941	3,565	303	8.5	<0.001
	Turkey/Morocco	69	58	2	3.4	
	Suriname/Antilles	250	203	26	12.8	
	Eastern Europe	148	108	5	4.6	
	Sub-Saharan Africa	44	39	2	5.1	
	Middle and South America	197	189	17	9.0	
	Asia	202	177	5	2.8	
	Other/unknown	492	437	54	12.4	
STI symptoms[c]	No	3,336	2,470	83	3.4	<0.001
	Yes	1,900	1,568	244	15.6	
	Unknown/missing	1,107	738	87	11.8	
Notified[c]	No	3,918	3,022	248	8.2	<0.001
	Yes	1,315	1,006	76	7.6	
	Unknown/missing	1,110	748	90	12.0	
Sexual preference	Bisexual	658	392	10	2.6	<0.001
	Homosexual	5,685	4,384	404	9.2	
Intravenous drug use	No/yes, ever	6,140	4,616	401	8.7	0.007
	Yes, in past six months	20	18	5	27.8	
	Unknown	183	142	8	5.6	
Commercial sex worker	No	6,154	4,541	408	8.8	0.164
	Yes	148	110	4	3.6	
	Unknown	41	25	2	8.0	
Client of commercial sex worker	No	6,243	4,716	412	8.7	0.193
	Yes	59	34	0	0.0	
	Unknown	41	26	2	7.7	
Number of sexual partners in past six months[d]	0–1	424	216	5	2.3	<0.001
	2–5	1,873	1,209	47	3.9	
	6–50	1,726	1,287	108	8.4	
	≥51	99	70	8	11.4	
	Unknown/missing	2,221	1,994	246	12.3	
Condom use in last sexual contact[e]	No	2,148	1,425	15	2.4	<0.001
	Yes	1,092	633	97	6.8	
	Unknown/missing	3,103	2718	302	11.1	
History of STI infection[f]	No	3,900	2,827	140	11.5	<0.001
	Yes	1,647	1,222	191	6.8	
	Unknown	796	727	83	11.4	
Previous HIV status	Negative	3,466	2,510	96	3.8	<0.001
	Positive	1,833	1,574	292	18.6	
	Unknown	1,044	692	26	8.9	
STI co-infections[g]	Gonorrhoea	1,418	1,060	107	10.1	0.061
	Infectious syphilis	398	292	37	12.7	0.012
	HIV (new infection)	307	244	11	4.5	0.018

CT: *Chlamydia* trachomatis; HIV: human immunodeficiency virus; LGV: lymphogranuloma venereum; MSM: men who have sex with men; STI: sexually transmitted infection.

[a] For one person, information on age was missing.

[b] Combination of two questions: self-defined origin (compulsory until 2010, voluntary in 2011) in which missing values for 2011 were filled with values for the new question: origin based on country of birth (voluntary in 2010, compulsory in 2011). Other/unknown: contained cases from other countries (in Europe) and cases with unknown origin.

[c] Optional question in 2007, compulsory since 2008.

[d] Optional question in 2006–09, compulsory question in 2010–11.

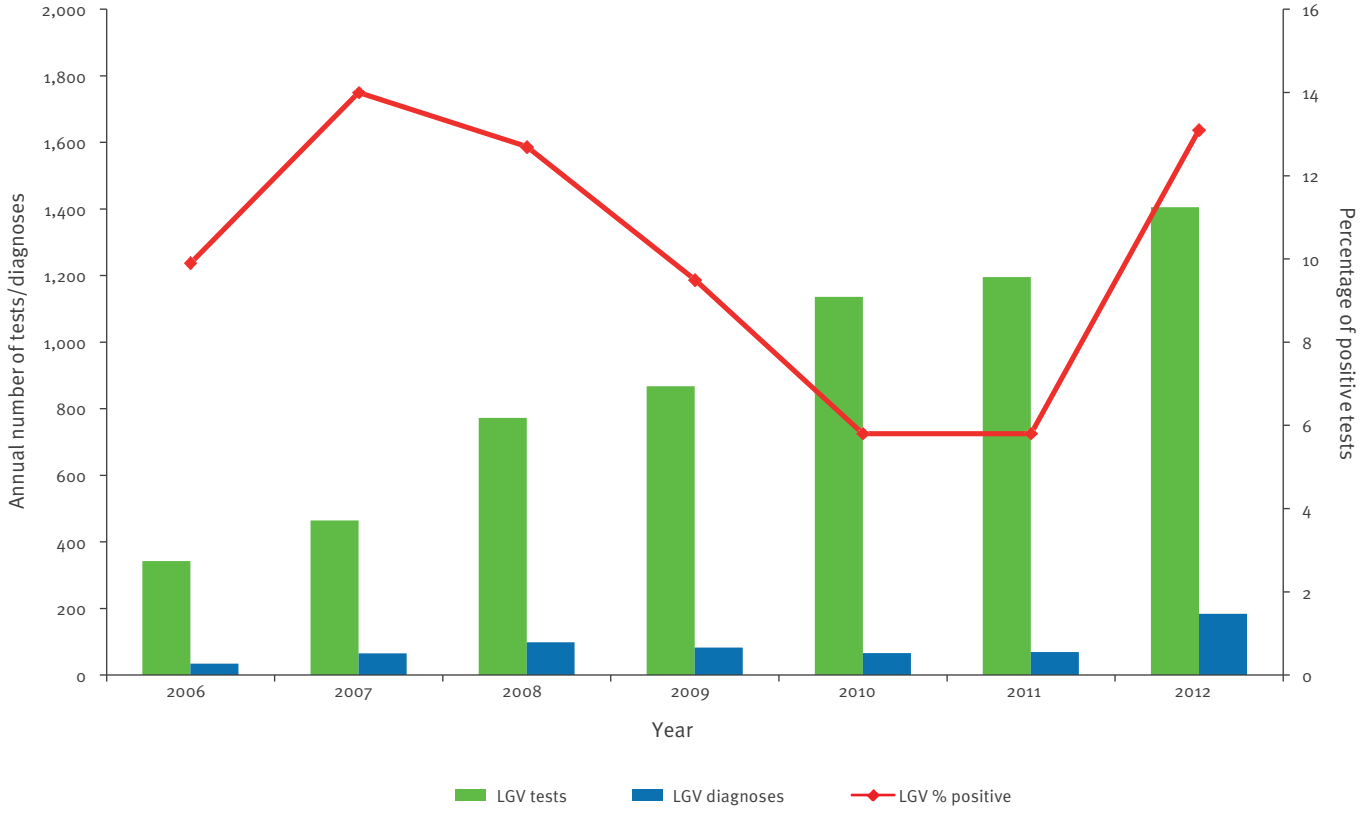
[e] Optional question 2006–2010, compulsory question in 2011.

[f] Gonorrhoea, chlamydia or syphilis infection in the past two years.

[g] Only those STIs are presented for which every client was tested.

Figure 1

Number of diagnoses (n=598), tests performed (n=6,181), and positivity rate for lymphogranuloma venereum, the Netherlands, January 2006–December 2012



LGV: Lymphogranuloma venereum.

The annual number of reported LGV cases among MSM tested for LGV started with 34 in 2006, and in 2011, 69 cases were reported by the STI clinics (Figure 1). Over this period, the annual number of reported cases fluctuated. Recent data from 2012 showed that 184 LGV cases were diagnosed in 2012.

The main differences between LGV-negative and LGV-positive MSM were in age and HIV status. Among anorectal chlamydia-positive MSM and MSM tested for LGV, approximately 15% were 40 to 44 years-old, whereas 26.3% (n=109) of LGV cases belonged to this age group (p<0.001). Furthermore, among MSM who were tested for LGV, 33.0% (n=1,574) were HIV-positive, and among LGV cases, 70.5% (n=292) were HIV-positive (p<0.001). Analyses of the characteristics of LGV cases by year showed no significant trends (data not shown).

Overall, 75.3% (n=4,776) of all cases of anorectal chlamydia were tested for LGV (Table 1). In 2010 and 2011, these were 85.8% (n=1,136) and 82.5% (n=1,195) respectively. Figure 2 illustrates that LGV testing rates differed per region, ranging from 6.5% to 98.3%.

Positivity rate

Over the period from 2006 to 2011 the overall positivity rate for LGV testing was 8.7% (Table 1). Since 2006, the annual number of LGV tests performed has steadily increased from 342 in 2006 to 1,195 in 2011 (Figure 1). Until 2008, the increase in the number of tests was reflected in an increase in the number of LGV cases diagnosed. After 2008, the number of tests increased while the number of cases diagnosed did not, resulting in a decreasing positivity rate from 2007 onwards, with 6% in 2010 and 2011. In 2012, a rise in positivity rate was seen (13.1%).

Risk factors

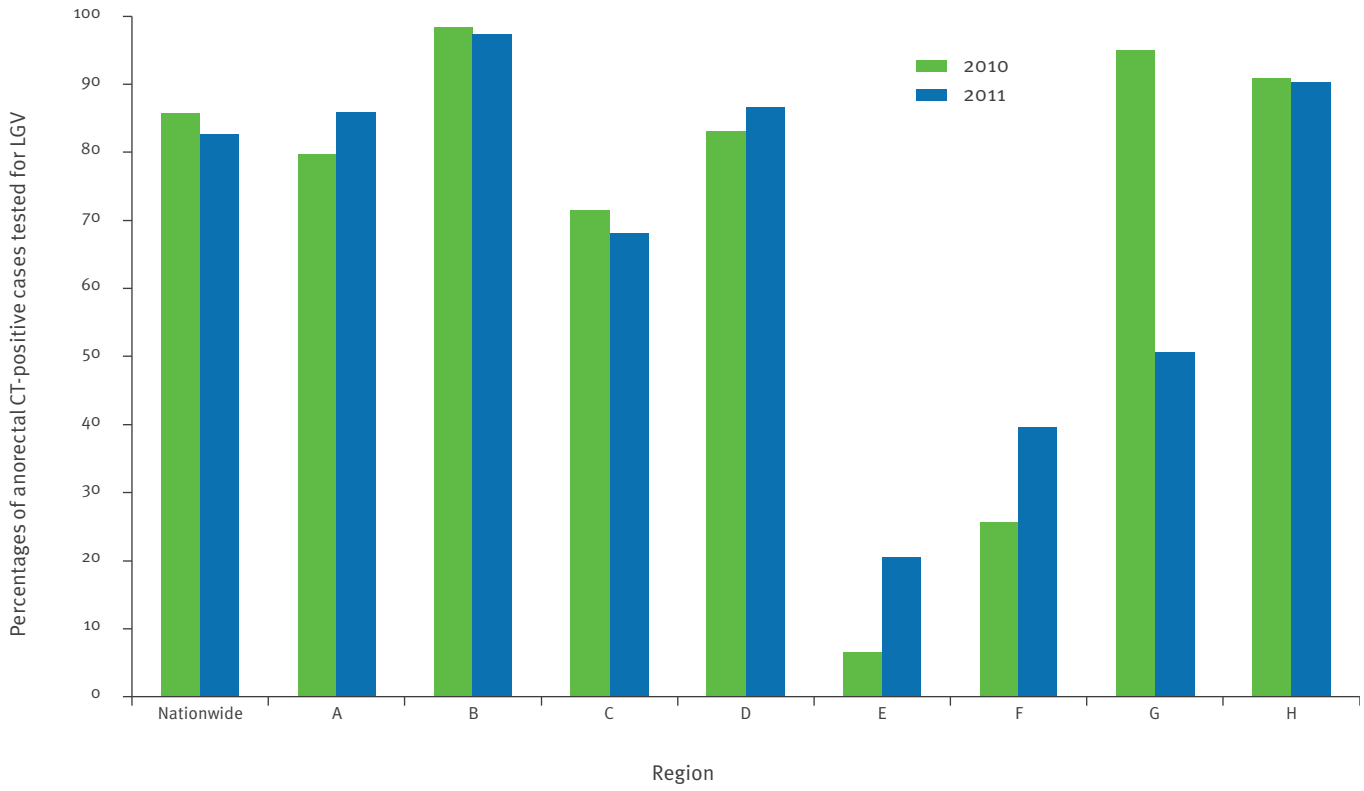
Risk factors for LGV infection were investigated using MSM who tested negative for LGV as the reference population (Table 2). The two risk factors for LGV infection with the highest odds ratios were HIV positivity (OR=4.1, 95% CI:3.2–5.3) and symptoms at the time of consultation (OR=4.1, 95% CI:3.1–5.3). Other identified risk factors for LGV were having had more than 50 sexual partners in the past six months (OR=3.7, 95% CI: 1.1–12.4), no condom use during last sexual contact (OR=2.2, 95% CI:1.2–3.9), homosexuality (as opposed to bisexuality; OR=2.2, 95% CI:1.1–4.2) and increasing age with a peak in the group aged 40–44 years (OR=2.1, 95% CI:1.5–2.8).

Underestimation of cases

By restricting the testing policy to a specific population of MSM positive for anorectal chlamydia, cases may be missed. Two strong risk factors identified in this

Figure 2

Geographical differences in lymphogranuloma venereum testing rates among anorectal Chlamydia-positive men who have sex with men, the Netherlands, 2010–2011 (n=6,343)



CT: *Chlamydia trachomatis*; LGV: lymphogranuloma venereum.

study were HIV positivity and clinical symptoms at the time of consultation (OR=4.1). If the testing policy for LGV was restricted according to either one or both of these factors, this would result in an underestimation of the actual number of cases. In the period from 2007 to 2011, 22.5% (n=87) of LGV cases were diagnosed among MSM who did not exhibit clinical symptoms, and 24.5% (n=95) of LGV cases were diagnosed among MSM who were HIV-negative (data on clinical was symptoms was not available before 2007). Moreover, in this period, 9.0% (n=35) of LGV cases were diagnosed among attendees who were neither HIV-positive nor exhibited clinical symptoms. Of the HIV-positive MSM among LGV cases, 21.8% (n=52) did not exhibit STI symptoms.

Combining data from 2010 and 2011, 2,719 cases of anorectal chlamydia were diagnosed among MSM. For 84.1% of these cases (n=2,286), LGV testing was performed. We estimated that 27 cases of LGV could have been missed in this period. With a recorded combined incidence over these two years of 136 cases, this would imply an underdiagnosis of 19.9%.

Discussion

The results indicate that while the incidence of LGV fluctuated during the period from 2006 to 2011, there was no clear increasing or decreasing trend. However, the reported incidence remained consistently higher than before the first outbreak in 2003 [5]. Data from 2012 showed a further increased number of reported LGV cases as well as a higher rate of positive tests. Since the proportion of MSM with an anorectal chlamydia infection tested for LGV remained stable over time (around 75%), the increase seems to be a real increase in positivity rate, rather than an increase due to more diagnostic testing. Although it remains unknown how the epidemic will develop in the future, current data underline the importance of active testing and continuous monitoring of the infection. After 2005, the annual number of LGV tests increased sharply, maybe due to widespread uptake and implementation of the RIVM and NVDV guidelines by municipal health centres. Until 2008, the number of LGV diagnoses also showed a slight increase, suggesting that implementation of these guidelines had improved case detection. It is therefore worrisome that in several regions LGV testing rates were low among MSM positive for anorectal chlamydia. This not only hampers national surveillance, but also detection and treatment of LGV infections.

The present study showed that older age (>40 years), clinical symptoms, sexual preference, having many partners in the past six months (>50), not using condoms, and HIV positivity remained the main risk

Table 2

Risk factors for LGV infection among MSM tested positive for LGV compared to MSM with a non-LGV anorectal *Chlamydia* infection at an STI clinic in the Netherlands, 2006–2011

Risk factors		Total Number	LGV-positive MSM			
			Univariate			Multivariate
			Number	OR (95% CI)	p value	OR (95% CI)
Total		**4,776**	**414**			
Year	2006	342	34	1.0	<0.001	1.0
	2007	464	65	1.5 (1.0–2.3)		1.1 (0.7–1.7)
	2008	772	98	1.3 (0.9–2.0)		0.7 (0.3–1.7)
	2009	867	82	1.0 (0.6–1.4)		0.6 (0.2–1.3)
	2010	1,136	66	0.6 (0.4–0.9)		0.3 (0.1–0.8)
	2011	1,195	69	0.6 (0.4–0.9)		0.3 (0.1–0.8)
Age group	<35	2,008	97	1.0	<0.001	1.0
	35–39	806	81	2.2 (1.6–3.0)		1.4 (1.0–2.0)
	40–44	774	109	3.2 (2.4–4.3)		2.1 (1.5–2.8)
	≥45	1,188	127	2.4 (1.8–3.1)		1.8 (1.4–2.4)
Origin[a]	The Netherlands	3,565	303	1.0	<0.001	NS
	Turkey/Morocco	58	2	0.4 (0.1–1.6)		
	Suriname/Antilles	203	26	1.6 (1.0–2.4)		
	Eastern Europe	108	5	0.5 (0.2–1.3)		
	Sub-Saharan Africa	39	2	0.6 (0.1–2.4)		
	Middle and South America	189	17	1.1 (0.6–1.8)		
	Asia	177	5	0.3 (0.1–0.8)		
	Other/unknown	437	54	1.5 (1.1–2.1)		
STI symptoms[b]	No	2,470	83	1.0	<0.001	1.0
	Yes	1,568	244	5.3 (4.1–6.9)		4.1 (3.1–5.3)
	Unknown/missing	738	87	3.8 (2.8–5.3)		1.7 (0.8–3.7)
Notified[b]	No	3,022	248	1.0	0.002	NS
	Yes	1,006	76	0.9 (0.7–1.2)		
	Unknown/missing	748	90	1.5 (1.2–2.0)		
Sexual preference	Bisexual	392	10	1.0	<0.001	1.0
	Homosexual	4,384	404	3.9 (2.1–7.3)		2.2 (1.1–4.2)
Intravenous drug use	No/yes,ever	4,616	401	1.0	0.025	NS
	Yes, past six months	18	5	4.0 (1.4–11.4)		
	Unknown	142	8	0.6 (0.3–1.3)		
Commercial sex worker	No	4,641	408	1.0	0.104	NS
	Yes	110	4	0.4 (0.1–1.1)		
	Unknown	25	2	0.9 (0.2–3.8)		
Client of commercial sex worker	No	4,716	412	1.0	0.044	NS
	Yes	34	0	0.0		
	Unknown	26	2	0.9 (0.2–3.7)		
Number of sexual partners in past six months[c]	0–1	216	5	1.0	<0.001	1.0
	2–5	1,209	47	1.7 (0.7–4.3)		1.3 (0.5–3.4)
	6–50	1,287	108	3.9 (1.6–9.6)		2.4 (0.9–6.2)
	>50	70	8	5.5 (1.7–17.2)		3.7 (1.1–12.4)
	Unknown/missing	1,994	246	5.9 (2.4–14.6)		1.8 (0.6–4.9)
Condom use in last sexual contact[d]	Yes	633	15	1.0	<0.001	1.0
	No	1,425	97	3.0 (1.7–5.2)		2.2 (1.2–3.9)
	Unknown/missing	2,718	302	5.2 (3.0–8.7)		2.4 (1.3–4.3)
History of STI infection[e]	No	2,827	191	1.0	<0.001	NS
	Yes	1,222	140	1.8 (1.4–2.2)		
	Unknown	727	83	1.8 (1.4–2.3)		
Previous HIV status	Negative	2,510	95	1.0	<0.001	1.0
	Positive	1,574	292	5.8 (4.6–7.4)		4.1 (3.2–5.3)
	Unknown	692	27	1.0 (0.7–1.6)		0.9 (0.6–1.5)

CI: confidence interval; HIV: human immunodeficiency virus; LGV: lymphogranuloma venereum; MSM: men who have sex with men; NS: not significant; OR: odds ratio; STI: sexually transmitted infection.

[a] Combination of two questions: self-defined origin (compulsory until 2010, voluntary in 2011) in which missing values for 2011 were filled with values for the new question: origin based on country of birth (voluntary in 2010, compulsory in 2011). Other/unknown: contained cases from other countries (in Europe) and cases with unknown origin.

[b] Optional question in 2006–07, compulsory since 2008.

[c] Optional question in 2006–09, compulsory question in 2010–11.

[d] Optional question in 2006–10, compulsory question in 2011 .

[e] Gonorrhoea, chlamydia or syphilis in the past two years. Optional question 2006–07, compulsory since 2008.

factors for LGV infection. These findings are similar to previous studies in which the majority of LGV infections were symptomatic, contrary to infection with other *C. trachomatis* serovars [13,14], and in which HIV seropositivity was a strong risk factor for infection [15,16]. Taken together, these observations suggest that LGV infection is still primarily located in a specific population.

That the two most used guidelines for LGV diagnosis and treatment at Dutch STI clinics recommend different testing policies for LGV, can have caused the diverging testing policies observed, which may have resulted in an underdiagnosis. As shown in the present study, restricting the testing policy according to, for example, the risk factors of showing symptoms and HIV positivity results in underdiagnosis. However, health benefits gained by increased detection will always need to be weighed against the costs for such testing. Additional research is needed into the consequences of a certain degree of underdiagnosis resulting from a restricted testing policy in the Netherlands.

In particular, it is not known whether a shorter treatment regimen would be sufficient to treat asymptomatic LGV infections. All people attending Dutch STI clinics are routinely tested for chlamydia. Since LGV is caused by *C. trachomatis* L serovars, cases are already prescribed treatment for this infection. The standard treatment regimen for anorectal chlamydia is one week of doxycycline. If the standard treatment regimen for non-LGV serovars were sufficient to treat asymptomatic LGV infections, the focus could shift towards the detection of symptomatic infections requiring additional treatment and the impact of the resulting underdiagnosis on the health situation in the Netherlands would be reduced.

STI surveillance is instrumental in monitoring the disease situation and contributes to the evaluation of implemented control strategies. Currently, STI clinics can prioritise their policy according to the local situation. This became evident by investigating LGV test rates for anorectal chlamydia-positive MSM in different regions, and was also evident in conversations with experts in the field. Different regions applied different testing criteria, and a consequence may be that national trends identified by surveillance are distorted and opportunities to interrupt transmission were missed. Furthermore, different guidelines provide different advice. Attempts to harmonise different guidelines are ongoing. A new version of the secondary care guideline is currently in development and is expected to be published at the end of 2013.

Since the start of the epidemic of LGV in 2003, the infection has remained limited to the MSM population. Spread of the infection to the general population, for example through bisexual MSM to heterosexual women, could be an important risk. There have recently been reports describing cases of rectal and bubonic LGV in women [17,18]. These reports indicate that expansion of LGV infection to other populations remains a realistic possibility. Therefore, it is important to remain vigilant for LGV infections also in heterosexual men and women with anorectal *Chlamydia* infections. Taken together, our results indicate that on the one hand, LGV mainly occurs in a specific population of MSM and it is only rarely observed among heterosexual males and females. On the other hand, it remains important to prevent and treat LGV in heterosexual men and women. Surveillance of these populations may allow to detect an expansion of the infection at an early stage.

A limitation of the present study is that LGV is not a notifiable disease in the Netherlands. Only data collected during STI consultations at STI clinics were available. However, reports from experts in the field suggest that a substantial proportion of LGV cases may be diagnosed at other sources of care such as hospitals. Another limitation is that the study was based on routinely collected surveillance data. Previous research shows that specific high-risk behaviour is an important risk factor for LGV infection [8]. Therefore, additional research could be performed to investigate in more detail which risk behaviours are risk factors for LGV infection among MSM in the Netherlands. If specific sexual practices result in increased risk, targeted prevention strategies could be developed.

Conclusion

Since LGV emerged as an STI among MSM in 2003, the incidence has been fluctuating around a level consistently higher than before the first outbreak. Over time, the infection has affected the same population as the one in which it originally emerged. Furthermore, recent case reports describe cases of LGV among women, and data for the first half of 2012 showed again an increased number of reported cases of LGV. Taken together, these observations underline the importance of tracing and monitoring the infection by strengthening and harmonising LGV diagnostic testing among MSM who are positive for anorectal chlamydia.

Dutch STI clinics

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Risk factors for *Chlamydia trachomatis* infection in adolescents: results from a representative population-based survey in Germany, 2003–2006

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Infections with *Chlamydia trachomatis* (CT) can lead to severe sequelae; however, they are not notifiable in Germany. We tested urine samples from participants of KiGGS (German Health Interview and Examination Survey for Children and Adolescents) for CT infections and linked the results to demographic and behavioural data from 1,925 participants (girls aged 15–17 years and boys aged 16–17 years) to determine a representative prevalence of CT infection in adolescents in Germany and to assess associated risk factors. Prevalence of CT infection was 2.2% (95% CI: 1.4–3.5) in girls and 0.2% (95% CI: 0.1–0.7) in boys. CT infection in girls was associated with higher use of alcohol, marijuana and cigarettes, lower social status, oral contraceptive use, pregnancy, repeated lower abdominal pain and higher rates of doctors' consultations within the preceding three months and consultation of gynaecologists within the last 12 months. In multiple logistic regression, we identified two predictors for CT infection: marijuana consumption often or several times within the last 12 months ($F(1,164)=7.56$; $p<0.05$) and general health status less than 'very good' ($F(1,164)=3.83$; $p=0.052$). Given our findings, we recommend enhancing sex education before sexual debut and promoting safe sex practices regardless of the contraceptive method used. Well-informed consumption of alcohol should be promoted, the risky behaviour of people intoxicated through consumption of marijuana highlighted and doctors' awareness of CT screening enhanced.

Introduction

Infections with *Chlamydia trachomatis* (CT) are the most frequently reported urogenital, bacterial sexually transmitted infections (STIs) worldwide. Latest figures (2005) from the World Health Organization (WHO) show that there were an estimated 101.5 million new cases per year of CT infection among adults aged 15 to 49 years [1].

In Europe, prevalence of CT infection among unscreened asymptomatic women ranges between 1.7% and 17% [2], with sexually active women and men under the age of 20 years and 25 years respectively being most affected. CT infections are asymptomatic in up to 90% of women and more than 50% of men [3]. Chlamydial infections can cause infertility in men and women and according to WHO, 10–15% of women with untreated infections develop symptomatic pelvic inflammatory disease or other severe sequelae such as infertility or extrauterine pregnancies [4]. However, the effect of screening programmes and intensified testing, CT persistence and natural history of CT infections are still under debate [5-10].

In Germany, infections with CT are not mandatorily reportable and no prevalence data exist at the national level. A laboratory reporting system exists only in one federal state, Saxony, where an increase in the number of reported infections has been observed, from 26.3 per 100,000 inhabitants in 2003 to 100.8 per 100,000 in 2009 [11].

Population-based studies have been performed in the United Kingdom (UK) (National Survey of Sexual Attitudes and Lifestyles, Natsal) [12], France (NatChla study) [13] and the United States (National Health and Nutrition Examination Survey, NHANES) [14] and prevalence of CT infection estimated. In the French study, which was performed among 18–29 year-olds, the prevalence in men was 2.5% (95% CI: 1.2–5.0) and 3.2% (95% CI: 2.0–5.3) in women. Infections were associated with last sex with a casual partner in both men and women, last sex with a new partner and living in Paris in men, and multiple partners in the last year, same-sex partners and low educational level in women [13]. In the UK study, sexual behaviour was assessed in 16–44 year-olds, such as age at first sex, contraception used at first intercourse, condom usage, pregnancy

and history of STIs. The highest prevalence of CT infection (3.0%; 95% CI: 1.7–5.0) was found in women aged 18–24 years. Factors associated with higher prevalence in both sexes were occurrence of first intercourse before the age of 16 years, main source of information about sexual matters being friends and not being sexually competent at first intercourse [12,15]. In the United States study, CT prevalence of 3.9% (95% CI: 2.2–6.9) was found in 14–19 year-old females and was – together with gonorrhoea, *Trichomonas* or herpes simplex virus 2 infection – associated with more lifetime sexual partners. The risk of infection was higher soon after sexual initiation [14].

Within KiGGS (German Health Interview and Examination Survey for Children and Adolescents), the general health of children and adolescents aged 0–17 years was mapped nationwide between May 2003 and May 2006. It was a population-based survey that collected health status data from 17,641 participants in 167 representative sites. The participation rate was 66.6% overall. Through a brief non-responder questionnaire, it was shown that the data collected were representative for the health status of adolescents in Germany [16]. Detailed sampling methods have been described previously by Kamtsiuris et al. [17].

In our study presented here, we retrospectively tested urine samples from 1,925 KiGGS participants (girls aged 15–17 years and boys aged 16–17 years) for CT infection and linked the results to the participants' demographic and behavioural data to determine a representative prevalence of CT infection in adolescents in Germany and to assess associated risk factors. To our knowledge, this is the first time that representative population-based CT-data have been linked to demographic and behavioural data in Germany. We have previously reported the results of a random sample of 12–17 year-old KiGGS participants that was tested for CT in pools of four [18]. However, no representativeness was assured due to this subsampling and we retested all urine specimens in pools of four as well as in single testing [19].

In our study, we estimated the prevalence of CT infection stratified by age and sex in Germany and identified risk factors (demographic, behavioural and health-related) associated with CT infection, representative for German adolescents. In addition, we assessed the usage of healthcare structures associated with CT infection.

Methods

Data collection in KiGGS included questionnaires filled in by parents and adolescents in parallel, an interview and a physical examination performed by a medical doctor. Biological specimens were taken and results could be linked to survey data. Urine samples were stored at –50°C and retrospectively tested for CT. To reduce costs and given our experience with the results from the previous random sample [18], we limited the age groups to be tested to 16–17 year-old boys and 15–17 year-old girls.

We used BD ProbeTec ET System (strand displacement amplification (SDA) system), a nucleic acid amplification test (NAAT), for pooled and single-specimen testing [19]. Testing was performed between January 2009 and February 2010. For the analyses shown here, we only used results from single-specimen testing.

We divided Germany into two geographical regions by federal state: 'East' (Brandenburg, Mecklenburg-Vorpommern, Saxony, Saxony-Anhalt, Thuringia and Berlin) and 'West' (Schleswig-Holstein, Hamburg, Bremen, Lower Saxony, North Rhine-Westphalia, Hesse, Bavaria, Rhineland-Palatinate, Saarland and Baden-Württemberg).

The size of a residential municipality was defined as 'rural' if it had fewer than 5,000 inhabitants, 'provincial' if between 5,000 and <20,000, 'urban' if between 20,000 and <100,000 and 'metropolitan' if 100,000 or more.

For comparison of behavioural factors, self-reported variables covering the last 12 months were grouped, such as the consumption of alcohol (beer, wine and hard liquor (schnapps)) into 'two glasses per week or more' and 'one glass per week or less'. Marijuana consumption was grouped as 'often or several times' (defined as 'frequent') and 'once or never'. Tobacco smokers were defined as currently being smokers; non-smokers were defined as participants who did not report smoking at the time of the survey. Frequent smokers were defined as participants who reported smoking daily, several times or at least once a week. Being frequently in smoky rooms was defined as being in the room daily, several times a week or at least once a week (the duration of stay in the room was not defined); the analysis was stratified by smoker status (smoker and non-smoker).

Variables such as socio-economic and migrant status were defined according to standardised procedures within KiGGS. A value for social status was calculated according to Winkler [20,21] using data on the education, occupation and net household income of parents. The social status index could vary between 3 and 21: values of 3–8 were defined as low social status, 9–14 as medium and 15–21 as high. As the methods used to calculate this index were based on data from the national adult health survey from 1998, they were adjusted for inflation and changes in educational systems using data from a telephone survey performed in 2003–04 [20].

The KiGGS participants were selected in a complex two-step sampling technique, based on a systematic sample of 167 study locations and local population registries. For all statistical analysis, we used sampling weights to adjust for sampling and study design-driven

Table 1

Urine specimens positive for *Chlamydia trachomatis* and weighted age- and sex-specific prevalence[a] of *C. trachomatis* infection, KiGGS participants[b], Germany, 2003–2006 (n=1,925)

Age in years	Female		Male	
	Number positive/ total number tested	Prevalence of CT infection (95% CI)	Number positive/ total number tested	Prevalence of CT infection (95% CI)
15	6/381	1.4 (0.6–3.3)	NA	NA
16	6/378	1.8 (0.6–4.9)	1/408	0.2 (0.0–1.3)
17	12/377	3.3 (1.8–6.1)	2/381	0.2 (0.1–1.0)
Total	**24/1,136**	**2.2 (1.4–3.5)**	**3/789**	**0.2 (0.1–0.7)**

CI: confidence interval; CT: *Chlamydia trachomatis*; KiGGS (German Health Interview and Examination Survey for Children and Adolescents); NA: not applicable.

[a] Weighted prevalence, as a percentage. As selected persons were representative for the general population, weighted proportions are reported. In addition, actual numbers are shown here to allow assessment of the size of the study and number of participants included in each subgroup.

[b] Samples tested were from 16–17 year-old boys and 15–17 year-old girls.

unequal probabilities of the participants being selected for the study [16]. As the selected persons were representative for the general population, apart from Table 1, we report (weighted) proportions, not actual numbers. .

Statistical analysis was performed using the Complex Samples Module in SPSS Statistics Version 17.0.3 and 18.0.0 (IBM SPSS, Chicago, IL, United States). For descriptive analyses of categorical data, frequencies and proportions were calculated. For measures of association chi-square and Fisher´s exact test were applied and odds ratios (ORs) with 95% confidence intervals (CIs) calculated. For continuous data, means were computed using the Complex Samples General Linear Model for linear regression analysis and Student's two-sample t-test. Statistical significance for all tests was set at the ‹0.05 level.

We performed multiple logistic regressions to assess risk factors for positive CT test results in girls aged 15–17 years. The criterion for inclusion in the multiple logistic regression model was having been statistically significant in a preliminary simple logistic regression, and as a result of this, boys were not included. Regression coefficients, Wald statistics and ORs with 95% CIs were assessed in two different models assessing behavioural risk factors and health(care)-related factors for the CT test results.

Data protection was assured, as no patient-identifying data were made available to us. Approval was granted by the Ethics Committee from University Hospital Charité in Berlin.

Results

Study population

We tested urine samples of 1,925 KiGGS participants: 789 boys aged 16–17 years and 1,136 girls aged 15–17 years. These numbers represent all study participants for whom urine samples were available. Overall, urine samples were collected for 92% of boys and 83% of girls in the respective age groups. We did not see a systematic bias in those who provided a urine sample, as there was no difference in their socio-demographic variables compared with those who did not provide a sample. The 1,925 participants included could be regarded as representative of the German population based on the geographical area of residence, the size of the residential municipality and social status (data not shown).

Prevalence of *C. trachomatis* infection

Overall, the weighted prevalence of CT infection was 2.2% among the girls tested and 0.2% among the boys (Table 1). The prevalence increased by age in the girls, being highest (3.3%; 95% CI: 1.8–6.1) in those aged 17 years.

Behavioural risk factors/indicators

No difference in geographical region of residence, size of residential municipality, migrant status or educational level could be found between CT-positive and CT-negative participants. Girls from low or medium social status were five times more likely to be CT-positive than girls from high social status (2.8% of girls from low or medium social status were CT-positive compared with 0.5% of girls from high social status (OR: 5.4; 95% CI: 0.9–31.4).

A large majority of participants reported that they consumed alcohol, there was no difference between CT-positive and CT-negative participants (100% compared with 92.5%). However, in 15 and 16 year-old girls, elevated consumption of alcohol (defined as two or more glasses per week), and frequent marijuana consumption (defined as often or several times) within the last 12 months was associated with CT infection, as shown in Table 2. Consumption of wine and hard

Table 2

Risk factors for *Chlamydia trachomatis* infection by *C. trachomatis* positivity and sex, KiGGS participants[a], Germany, 2003–2006 (n=1,925)

Risk factor	Age in years[c]	Female			Male[b]	
		Percentage CT positive[d]	Percentage CT negative[d]	Unadjusted OR (95% CI)[d]	Percentage CT positive[d]	Percentage CT negative[d]
Alcohol consumption (≥2 glasses/week)[e]	15	22.0	9.9	2.6 (0.3–24.2)	NA	NA
	16	73.3	16.9	13.5 (2.7–66.7)	100	48.4
	17	9.4	21.6	0.4 (0.0–3.0)	0	57.9
Marijuana consumption (often/several times)[f]	15	20.6	1.9	13.1 (1.3–133.2)	NA	NA
	16	43.0	5.3	13.4 (1.2–149.0)	0	9.3
	17	16.0	7.4	2.4 (0.5–12.6)	0	12.1
Tobacco smoking						
Smokers	NA	68.5	35.0	4.0 (1.6–10.0)	43.1	43.0
Mean number of cigarettes smoked daily	NA	13.3	7.8	p<0.02	22.0	9.6
Mean age when started smoking	NA	13.6 years	13.9 years	p=0.53	11.0 years	14.2 years
Being frequently in smoky rooms[g]						
All participants	NA	91.6	66.2	5.6 (1.6–19.4)	65.0	73.4
Smokers only	NA	90.0	81.0	2.1 (0.4–10.2)	100	84.1
Non-smokers only	NA	94.2	58.4	11.7 (1.4–97.9)	38.6	64.8

CI: confidence interval; CT: Chlamydia trachomatis; KiGGS (German Health Interview and Examination Survey for Children and Adolescents); NA: not applicable; OR: odds ratio.

[a] Sample comprised 16–17 year-old boys and 15–17 year-old girls.

[b] Due to small numbers and lack of statistical significance, no OR is shown for boys.

[c] Applicable for consumption of alcohol and marijuana only. For tobacco smoking, the participants were analysed by the respective age group (see footnote a).

[d] Weighted percentage, unless otherwise specified (as in Mean number of cigarettes smoked daily and Mean age when started smoking, where significance was calculated using Complex Samples General Linear Model for linear regression analysis). As selected persons were representative for the general population, (weighted) proportions, not actual numbers, are reported.

[e] Consumption of two or more glasses per week in comparison with one glass per week or less.

[f] 'Often/several times' in comparison with 'once/never'.

[g] Defined as being in the room daily, several times a week or at least once a week (the duration of stay in the room was not defined).

liquor was particularly associated with CT infection in the 15–16 year-old girls (data not shown).

Of all the participants tested, 66.6% of those who were CT-positive and 38.5% of those who were CT-negative smoked tobacco (OR: 3.2; 95% CI: 1.4–7.3). The mean number of cigarettes smoked daily was 13.9 in those who were CT-positive and 8.7 in those who were CT-negative (p=0.02). A total of 89.7% of CT-positive and 69.3% of CT-negative participants reported being in a smoky room at least once a week (OR: 3.9; 95% CI: 1.3–11.3).

Oral contraceptive use was associated with CT infection. Girls taking them were three times more likely to be CT-positive than those who did not (4.1% vs 1.3%; OR: 3.2; 95%CI: 1.1–8.7). In 15 year-old girls, CT-positivity was 5.3% in those who took oral contraceptives and 0.8% in those who did not (OR: 7.3; 95% CI: 1.3–42.1). In 16 year-old girls, it was 4.6% in those using oral contraceptives and 0.5% in those who did not (OR: 9.4; 95% CI: 0.9–96.7).

A total of 5% of all CT-positive girls were pregnant, compared with 0.1% of all who were CT-negative (OR: 91.1; 95% CI: 5.3–1,560.7). Vice versa, of all pregnant girls, 65.8% were CT-positive compared with a prevalence of 2.1% in non-pregnant girls.

A multiple logistic regression analysis was performed using CT-positive status as outcome, three health behaviour predictors (frequent marijuana consumption within the last 12 months, being frequently in smoky rooms and use of oral contraceptives), as well as one social class predictor. As shown in Table 3, according to the Wald criterion, only frequent marijuana consumption reliably predicted CT-positivity (Wald-F(1,164)=7.56; p<0.05). The OR indicates that girls frequently smoking marijuana are six times more likely to be CT-positive than those who do not frequently

Table 3

Multivariate logistic regression models of *Chlamydia trachomatis* prevalence in 15–17 year-old girls as function of behavioural risk factors and health(care)-related factors, KiGGS participants, Germany, 2003–2006 (model 1, n=1,041; model 2, n= 945)[a]

Factor	B	Wald-F	OR (95% CI)
Model 1: Behavioural risk factors			
Social class	−1.63	2.88	0.20 (0.03–1.31)
Frequent marijuana consumption	1.88	7.56	6.54 (1.70–25.18)
Frequent exposure to smoky rooms	1.47	3.53	4.36 (0.93–20.53)
Oral contraceptive use	0.71	1.30	2.03 (0.59–6.94)
(Constant)	−5.18	64.93	NA
Model 2: Health(care) factors			
General health status 'very good'	−2.07	3.83	0.13 (0.02–1.02)
Repeated episodes of lower abdominal pain within last 3 months	0.78	1.92	2.2 (0.72–6.70)
Gynaecologist visit within last 12 months	1.56	3.40	4.77 (0.90–25.46)
(Constant)	−5.23	77.82	NA

B: regression weight; CI: confidence interval; KiGGS (German Health Interview and Examination Survey for Children and Adolescents); NA: not applicable; OR: odds ratio.

[a] The fewer numbers are due to missing data.

smoke marijuana. Although being frequently in smoky rooms was not significantly identified as a predictor of CT-positivity (F(1,164)=3.53; p=0.06), its effects need to be assessed through larger studies.

Health(care)-related factors

All CT-positive participants were less likely to rate their general health status as 'very good', compared with those who were CT-negative, 2.5% vs 21.0% (OR: 10.5; 95% CI: 2.3–47.6). CT-positive girls additionally reported more frequently repeated episodes of lower abdominal pain within the last three months than CT-negative girls did, 45.5% vs 23.5% (OR: 2.7; 95% CI: 1.0–7.3).

Participants with CT more often consulted a doctor within the last three months, (82.2% vs 63.2%; OR: 2.7; 95% CI: 1.1–6.8). In girls, this difference was also seen in those visiting a gynaecologist within the last 12 months, 81.1% in those who were CT-positive compared with 46.0% in those who were CT-negative (OR: 5.0; 95% CI: 1.4–17.9).

The multiple logistic regression analysis to predict CT-positive status in girls showed that only general health status 'very good' significantly improved the model (F(1,164)=3.83, p=0.052). The OR and regression weight indicated that having a general health status that was not considered 'very good' increased the chance of being CT-positive. The other two predictors of the logistic regression model were repeated episodes of lower abdominal pain within the last three months and a gynaecologist visit within the last 12 months. According to the Wald criterion, the ladder variable did not significantly predict a positive CT status. (F(1,164)=3.40, p=0.07). However, the OR indicates an almost five times higher chance of being CT-positive if the girl had been to a gynaecologist within the last 12 months. Another possible predictor of the model that was not significant (F(1,164)=1.92; p=0.17) was repeated episodes of lower abdominal pain, but the OR showed a more than double increased chance of CT infection.

Healthcare structures

Overall, adolescents visited the following medical specialists at their last consultation: general practitioner 42.4%, paediatrician 12.6%, dermatovenerologist 6.6% or gynaecologist 8.5%. The medical specialty at the last visit by CT-positivity and sex is shown in Table 4.

Discussion

In this first, representative, population-based study of adolescents in Germany, we found an increase in the prevalence of CT infection with age in girls. As participants were unaware of their test results when filling out the questionnaire, we consider that their responses were unbiased. The estimated prevalence was particularly high in those who reported oral contraceptive use or who were pregnant. Independent predictors of CT infection were found to be frequently smoking marijuana and a general health status less than 'very good'. Social status, consumption of alcohol or marijuana and smoking were associated with CT- infection rates. Repeated lower abdominal pain was associated

Table 4

Medical specialty visited at last consultation by *Chlamydia trachomatis*-positivity and sex, KiGGS participants[a], Germany, 2003–2006 (n=1,925)

Medical specialty visited at last consultation	Female			Male[b]	
	Percentage CT positive[c]	Percentage CT negative[c]	Unadjusted OR (95% CI)	Percentage CT positive[c]	Percentage CT negative[c]
General practitioner	44.3	40.7	1.2 (0.5–2.8)	22.0	49.1
Gynaecologist	24.3	15.0	1.8 (0.7–4.6)	0.0	0.0
Ophthalmologist	15.7	4.5	4.0 (0.8–19.8)	0.0	3.7
Paediatrician	9.9	12.4	0.8 (0.2–2.9)	35.0	12.8
Dermatovenerologist	0.0	6.3	NA	43.1	7.1
Other[d]	5.8	21.1	0.2 (0.1–1.0)	0.0	27.2

CI: confidence interval; CT: *Chlamydia trachomatis*; KiGGS (German Health Interview and Examination Survey for Children and Adolescents); NA: not applicable; OR: odds ratio.

[a] Sample comprised 16–17 year-old boys and 15–17 year-old girls.

[b] Due to small numbers and lack of statistical significance, no OR is shown for boys.

[c] Weighted percentage. As selected persons were representative for the general population, (weighted) proportions, not actual numbers, are reported.

[d] Comprised internist, orthopaedist, otorhinolaryngologist (ear, nose and throat doctor), neurologist/psychiatrist, psychologist, surgeon, radiologist, urologist, school physician, other doctor.

with CT infection in girls and infected girls consulted a gynaecologist more often than CT-negative girls did.

An estimation of sexual experience in German youth has been reported through another representative national study, a telephone survey performed by the Federal Centre for Health Education (BzgA) during the same time period [22]. In this study, 2,500 randomly chosen adolescents aged 14–17 years were asked about their attitudes and behaviour concerning sexuality and contraception. Some 23% of the 15 year-old girls were sexually experienced, reaching 73% in 17 year-old girls. A total of 35% and 66% of 16 and 17 year-old boys respectively were sexually experienced. Taking these results into account, our findings showed the highest prevalence of CT infection (6.8%) in 15 year-old girls who were presumably sexually experienced. Overall, the prevalence in girls aged 15–17 years was 4.4% and 0.8% in 16–17 year-old boys. However, participants in both surveys were probably not identical, limiting further analysis.

A 2.2% prevalence of CT infection in all girls aged 15–17 years in our study in Germany was lower than the 3.9% found by Forhan et al. in females aged 14–19 years in the United States [14]. The highest prevalence we found, in 15 year-old girls presumed to be sexually experienced (6.8%), was comparable to the 7.1% found in sexually experienced female NHANES participants in the United States [14]. Most other population-based studies were performed in sexually experienced participants aged 18–24 years, showing among women a prevalence of 3.6% (95% CI: 1.9-6.8) in France, 4.7% (95% CI: 2.5–8.5) in Slovenia and 3.0% (95% CI: 1.7–5.0) in the UK [12,13,23]. In an observational study performed in 2004 among sexually experienced females aged 14–20 years in Berlin, Germany, CT-positivity of 6.5% (95% CI: 4.7–9.0) was found [24]. In a study performed in 2008–09 in a mid-sized town in Germany, a prevalence of 4.2% was found in 14–19 year-old females and was associated with an early age of first sexual contact and increasing number of lifetime sexual partners [25]. The prevalence in our study might have been underestimated due to the long storage time between taking of specimens being taken and testing. In addition, if the collected urine was not first-void, there could be a reduction in sensitivity [3,26,27].

In our study, prevalence of CT infection was higher in girls than in boys. Men in general are reported to have CT infections at an older age than women [28, 29]. The reason could be that (younger) females tend to have sex with older males, as shown in previous studies [30, 31]. Bridging by age (defined as having sexual partners in more than one age group) was a predictor for reduced condom use, probably due to differences in power to make decisions on contraceptive use with older partners [30,32]. Furthermore, 'age-bridgers' engaged in more risky sexual behaviour in a cohort of CT-positive heterosexual young men aged 14–24 years [33]. Other reasons for higher prevalence in young girls could be cervical ectopy [34], earlier sexual debut or more partners during this earlier period of sexual experience [23,25].

Poor healthcare-seeking behaviour associated with higher infection rates, lower partner referral or inadequate care have been reported for people with lower socio-economic status in many countries [35-37]: our findings of a higher prevalence in girls with low or medium social status are therefore not surprising. In Germany, a quarterly fee has to be paid by persons insured by one of the general health insurances in order to access healthcare, posing a possible barrier for people with a small income. Therefore the higher prevalence in groups with low or medium social status could be due to a lower healthcare use and subsequently a lower chance to be tested for CT [38-40].

Although the number of participants in our study was small, we found an association between alcohol consumption and infection, particularly in younger girls. Excessive consumption of alcohol has been reported in association with an increase in sexual encounters and multiple (new) sexual partners and engagement in (unprotected) casual sex [41, 42]. In a study performed among female Irish students, consumption of alcohol by the person themselves or by their partners was the most frequent cause of unprotected sex [43]. Alcohol consumption can lead to failure in condom usage, such as breakage or falling off, and was positively associated with infection if male partners had drunk alcohol [44,45].

We found that tobacco smoking was associated with CT infection, similar to the findings of a study among women aged 18–25 years in Costa Rica [46]. The immunosuppressive effect of cigarette smoking on the cervical epithelium – due to a decrease in the concentration of Langerhans' cells and hence reduced ability to present viral antigens to T lymphocytes and subsequent persistence of local viral infection and the increased likelihood of the development of a virally induced neoplastic transformation – has been shown for human papillomavirus previously [47]. Smoke-induced persistent *C. pneumoniae* infections have been observed in endothelial cells [48]. Whether there is a biological effect of smoking on CT infection is, however, unknown and warrants further investigation. In our studied population, it is also possible that smoking was associated with an active nightlife, resulting in smoking and drinking with peers and more frequently engaging in casual sex; unfortunately, however, there was no systematic gathering of this information in the survey. Adolescents who reported being frequently in smoky rooms might also be from families with lower social status and hence smoking might be a confounder. Nevertheless, a dose–response relationship in the number of cigarettes smoked per day supports some effect of smoking in our study.

An association between the consumption of marijuana and CT infection was found in African-American adolescents [49]. In a representative survey performed by BzgA in 2011, no association between taking illegal drugs – dominated by marijuana in Germany – and social or educational status was found [50]; hence, social class might have little influence on marijuana consumption in Germany.

Oral contraceptive use and pregnancy was positively associated with CT infection in our study, as previously reported [34,45,51]. One explanation could be that young girls who take the pill no longer use condoms [24], as pregnancy prevention has been frequently reported as a major reason for condom use in young females. In the United States, an increase in oral contraceptive intake from 8% in 9th-grade females to 30% in 12th-grade females was found as was a concomitant decrease in condom use from 56% to 49% in those sexually active females [52]. We do not know if the pregnancies in our study were unwanted; however, in the UK, which has the highest teenage-pregnancy rates in Western Europe [53], CT infection rates are very high in young girls too, with 3,027 reported infections per 100,000 population in females aged 15–19 years in 2011 in England [54]. In our study, the number of pregnant girls was very low, and hence the confidence intervals were very wide.

CT-positive girls more frequently reported lower abdominal pain than CT-negative girls. These symptoms may be related to CT infection as lower abdominal pain has been described in acute CT infection and also in patients with pelvic inflammatory disease [55]. In our study however, we do not know whether participants were aware of a possible previous untreated CT infection or the detailed time frame of pain. Also, lower abdominal pain can have many causes and it is therefore difficult to establish a causal link between lower abdominal pain and CT infection. Even if the pain was linked to CT infection, physicians might not have considered CT infection and might not have taken their sexual history. Our study was performed in 2003–2006 and CT screening for young sexually active women was only introduced in 2008 in Germany. Therefore, physicians were unable to test asymptomatic persons free of charge at that time and consequently might have missed some CT infections.

Currently, annual CT screening for sexually active women under the age of 25 years can be performed using urine samples [56] and it is common practice for this to be done at gynaecologists' practices; however, the extent of screening uptake is currently unknown in Germany. From our study, we recommend extending the screening guidelines to other specialties in Germany, such as GPs, paediatricians and dermatovenerologists, as in our study, the majority of adolescents attended these doctors and as urine can easily be collected in these practices.

There are several limitations of our study. First, no data on sexual activity were collected; estimation of sexual activity had to be derived from another national representative study, thus posing a risk of uncertainty. However, as both surveys were representative,

behavioural data from the other representative survey were applied to our participants. Second, lower abdominal pain was reported by participants and not verified by clinical examination during the study visit. Similarly, information on most other risk factors analysed were self-reported and recall-bias or social expectancy is possible. However, as participants were unaware of their CT-infection status, this bias is likely to be equally distributed. Third, as the number of urine samples positive for CT was low, this led to wide confidence intervals in the univariate and multivariable analysis. Therefore, it is possible that more risk factors would have been identified if there had been a larger sample size or higher prevalence. Nevertheless, we believe that the results presented in this paper are valuable, as it is very difficult to obtain such a large, representative sample size.

Recommendations

To reduce CT prevalence, particularly in young girls, a number of interventions need to take place in Germany.

- Sex education needs to be enhanced before sexual debut and safe sex practices promoted with all new partners, regardless of the contraceptive method used.
- All sexually active girls and boys should be offered a CT test when attending a medical doctor. Those tested positive should be treated adequately, regardless of whether or not they have symptoms, to reduce the prevalence in the community. Asymptomatic men represent a reservoir for CT, potentially leading to females becoming infected. In a study of asymptomatic couples in Germany in 1996 using ligase chain reaction, more infections were detected in urine specimens from males than from females [57]: this supports the inclusion of men in CT screening. It has been shown that screening coverage needs to increase up to 26–43% to bring about substantial reductions of CT prevalence [58].
- Social discrimination needs to be reduced by ensuring uniform access to the healthcare system.
- Well-informed consumption of alcohol should be promoted and the risky behaviour of people intoxicated through consumption of marijuana highlighted through targeted adolescent health campaigns.
- Doctors should be made more aware of the need to test for CT and continuing sexual health education for doctors adopted. Screening should be extended to other specialties , so that urine samples for testing are taken at all consultations, not just those of gynaecologists. A GP's surgery can be an ideal place for CT screening, as adolescents perceive it as a normal place to discuss health issues [59] and hence can particularly be used to increase testing in boys.
- Appropriate models should be created for payment of counselling, particularly regarding contraception and sexual behaviour.

For the next round of this population-based survey starting at the end of 2013, it is encouraged to test specimens for CT shortly after sampling to reduce possible loss in sensitivity due to long storage. In addition, basic questions regarding sexual behaviour should be included. Finally, repeated cross-sectional surveys on a representative sample of German youth will provide information on possible changes in prevalence and risk behaviour and hence allow revaluation of recommendations.

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Conflict of interest

None declared.

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