Impact factor 5·9

# Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 22 | Weekly issue 5 | 02 February 2017

## Rapid communications



## Surveillance report



## Research Articles



ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

# Ongoing outbreaks of hepatitis A among men who have sex with men (MSM), Berlin, November 2016 to January 2017 – linked to other German cities and European countries

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**Citation style for this article:**
Werber D, Michaelis K, Hausner M, Sissolak D, Wenzel J, Bitzegeio J, Belting A, Sagebiel D, Faber M. Ongoing outbreaks of hepatitis A among men who have sex with men (MSM), Berlin, November 2016 to January 2017 – linked to other German cities and European countries. Euro Surveill. 2017;22(5):pii=30457. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.5.30457

Article submitted on 20 January 2017 / accepted on 02 February 2017 / published on 02 February 2017

**Since 14 November 2016, 38 cases of hepatitis A have been notified in Berlin; of these, 37 were male and 30 reported to have sex with men (MSM). Median age of MSM cases is 31 years (range: 24–52 years). Phylogenetic analysis revealed three distinct sequences, linking cases in Berlin to those in other German cities and to clusters recognised in other European countries in 2016.**

On 14 December 2016, the local public health authority (LPHA) of the Berlin district Mitte informed the State Office for Health and Social Affairs (SOHSA) in Berlin, of two male cases of hepatitis A, notified in calendar week 50, who identified themselves as men who have sex with men (MSM). At that time, no increase in hepatitis A cases was apparent in the notification data.

Immediately following this information, we enhanced epidemiological and virological surveillance of hepatitis A in Berlin and report here preliminary findings.

## Enhanced surveillance and molecular analyses

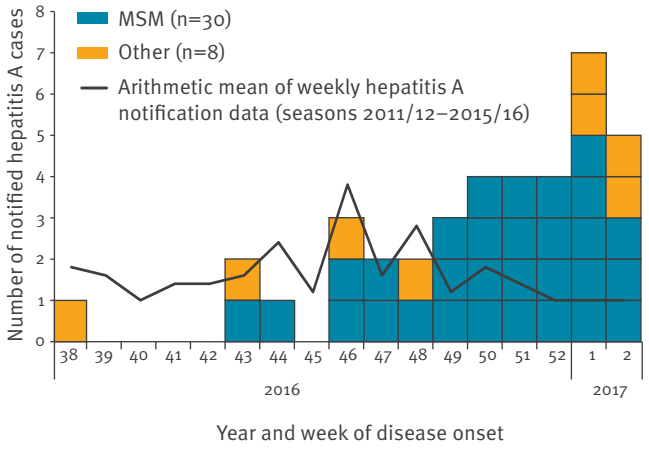
In the absence of an increase of hepatitis A in the notification data of Berlin in calendar week 50/2016, we (arbitrarily) considered a possible outbreak beginning as of calendar week 46/2016 (starting 14 November), i.e. four weeks (mean incubation period of hepatitis A) before the hepatitis A cases in MSM were first recognised. This coincided with when notified hepatitis A cases started to be predominantly male adults. We applied the case definition that is also used for surveillance purposes in Germany, i.e. symptomatic disease defined as fever or one of the following: abdominal discomfort, increase in serum transaminases, jaundice, plus laboratory confirmation, i.e. detection of hepatitis A virus (HAV) nucleic acid or HAV-specific IgM or a distinct increase in IgG [1]. We requested all 12 LPHAs in Berlin to systematically collect additional information on hepatitis A cases, notified as of calendar week 46/2016, in a specifically designed spreadsheet, including information on sexual contacts, sex in non-household venues and drug use, during their assumed period of infection. SOHSA collated case information submitted electronically by LPHAs.

LPHAs were also asked to organise sequencing of hepatitis A virus (HAV) from IgM positive serum samples or stool samples of cases notified as of calendar week 50 at the National Consultant Laboratory for Hepatitis A and Hepatitis E in Regensburg. Nucleic acid isolation, quantitative reverse transcription PCR (RT-qPCR) and sequencing were conducted as described elsewhere [2]. Sequencing primers were chosen according to the HAVNET unified typing protocol [3]. We queried GenBank for sequences with high similarity using the BLAST algorithms. A rooted maximum likelihood phylogenetic consensus tree for sequences of a 437 nucleotide (nt) long fragment in the VP1/P2A junction region was inferred using MEGA version 7.0.18 software.

In order to obtain information about possibly linked cases in other European Union countries, we communicated the information about the increase of hepatitis A in Berlin together with sequence information via the European Centre for Disease Prevention and Control

**Figure 1**

Notified cases of hepatitis A, stratified by sexual orientation and sex by week of symptom onset, Berlin, Germany, 14 November 2016–20 January 2017 (n=38)




(ECDC)'s Epidemic Intelligence Information System (EPIS) for food- and waterborne diseases and zoonoses (FWD) and the EPIS for sexually transmitted infections.

## Description of the outbreak

As at 20 January 2017, 38 cases of hepatitis A have been notified in Berlin since 14 November 2016 (calendar week 46). Of these, 37 are male, and one is female (Table).

Sexual orientation is known for 32 cases (31 men, one woman); 30 identified themselves as MSM, one as heterosexual and the woman as having sex with women (WSW). Median age of the 30 MSM cases is 31 years (range: 24–52 years); they live in seven of the 12 districts in Berlin, and most of them in Mitte (n=10). Disease onset of MSM cases ranges over an 11-week period (calendar weeks 43/2016–2/2017, Figure 1), which is incompatible with a common exposure to a point source. Three cases are epidemiologically linked to three other notified cases, supporting the assumption of transmission by interpersonal spread. Six cases have a travel history outside Germany (Spain (n=2), Austria, Greece, Malta, Taiwan (n=1 each) during the assumed period of infection, but the majority was apparently infected in Germany (likely in Berlin).

None of the MSM cases reported intravenous drug use. One MSM case was vaccinated with one dose of a monovalent hepatitis A vaccine 11 months before disease onset (a second dose within 6 to 12 months after the first dose is usually recommended by manufacturers to provide long-term protection); all others for which information on vaccination is available (n=27) were unvaccinated (n=23) or their vaccination was incomplete (n=3, single doses of HAV/HBV combination vaccine or unknown vaccine more than one year before disease onset) or outdated (n=1, last dose in 2001).

Sequencing results and phylogenetic analysis show three distinct clusters of MSM-related HAV strains currently circulating in Berlin (Figure 2).

The five sequences in the cluster Ber/Muc/Fra (including the WSW) are identical (100% match in the investigated 437 nt long fragment) to the HAV strain first observed in a MSM patient in August 2016 in Munich and later in a MSM patient in Frankfurt (prototype sequence V16–25801). The HAV sequences of three cases in the cluster Ber/NL are identical to the previously reported MSM-related HAV sequence RIVM-HAV16–090, which was isolated from two patients in September 2016, who had visited the EuroPride in Amsterdam in August 2016 [4]. Two of the identified cases fit in the third cluster Ber/UK with also identical sequences as compared with the MSM HAV outbreak strain UK VRD 521 circulating in the United Kingdom (UK) and reported in 2016 [4]. The closest match in the National Center for Biotechnology Information (NCBI) sequence database for the Ber/Muc/Fra cluster was isolated in 2013 in Italy during a multi-country European food-borne outbreak (IZSLER-005, acc. KU570286.1, 99.5% identity) [5], matches for the other clusters are described in [4].

Through EPIS-FWD, colleagues from Austria, Denmark and the Netherlands reported sporadic cases with sequence identity to the Ber/Fra/Muc-Cluster, some of which reported having sex with men in Berlin before disease onset.

## Background

HAV is predominantly transmitted via the faecal-oral route through person-to-person contact or contaminated food and water. The mean incubation period is 28 days (range: 15 to 50 days). Infected persons are most likely to transmit HAV before the onset of jaundice, when HAV concentration in stool is highest [6]. Transmission through sexual contact, particularly in MSM [7] as well as through sharing of needles and syringes has also been described [8]. Hepatitis A is a vaccine preventable disease and the German Standing Committee on Vaccination recommends vaccination of people with sexual behaviour at high-risk for HAV infection (such as homosexual contacts) [9]. Recommended vaccinations are paid for by health insurances in Germany.

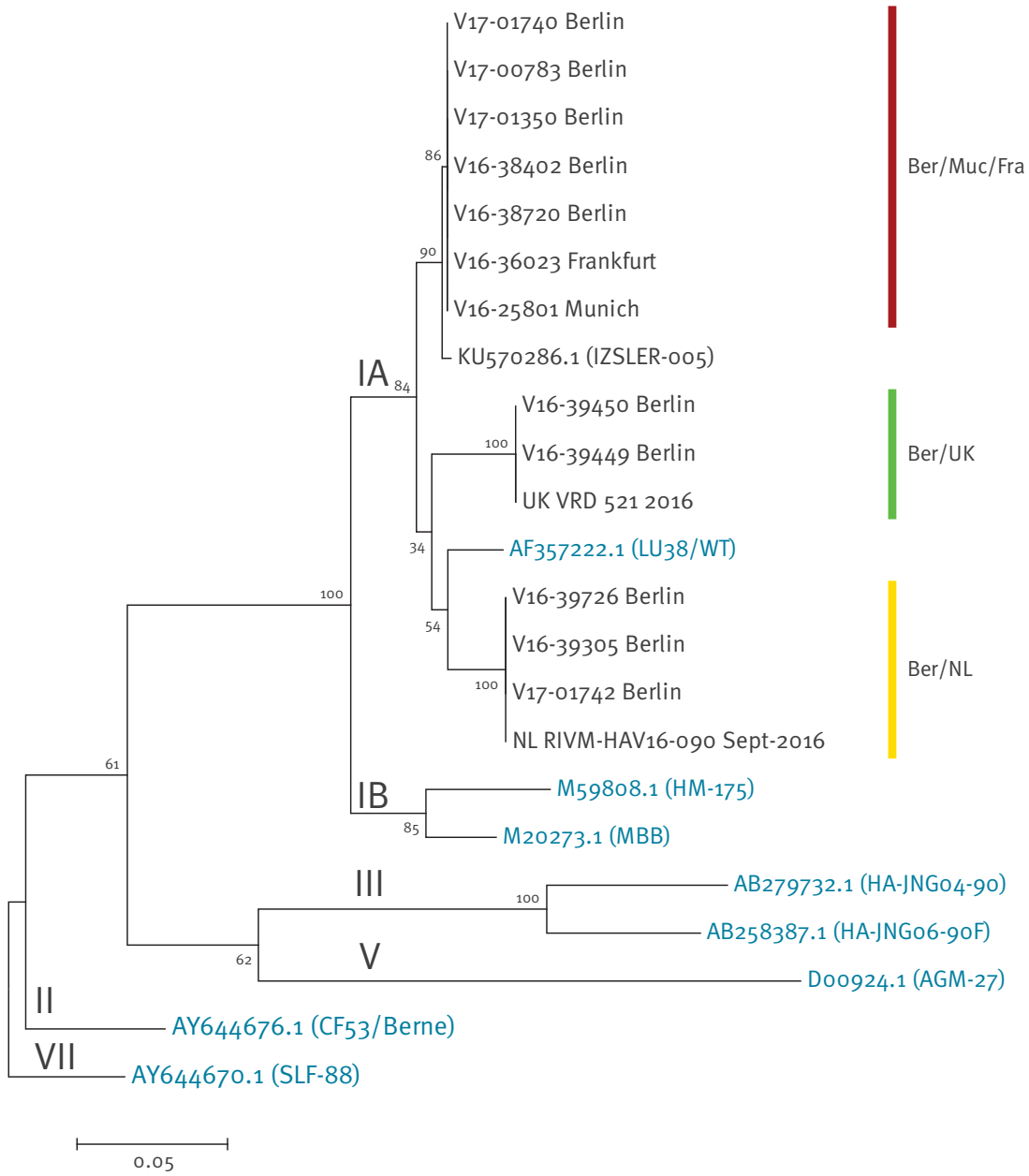
Germany is a low incidence country with 0.9 notified cases per 100,000 population in 2016. Virtually all HAV infections are directly or indirectly imported [10].

## General and specific public health measures in Germany

In response to the present outbreak, LPHAs educated cases about personal hygiene, traced cases and their contacts and recommended vaccination or post-exposure prophylaxis to contacts according to their risk profile. In addition, LPHA's, the SOHSA and the Robert Koch Institute (RKI, German national public health institute) formulated prevention recommendations to

**Figure 2**

Phylogenetic analysis of hepatitis A viruses, outbreak among men who have sex with men, Berlin, Germany, 14 November 2016–20 January 2017




HAV: hepatitis A virus.

Molecular Phylogenetic analysis of the VP1/P2A junction region of selected HAV isolates by Maximum Likelihood method. Genotype VII was used as an outgroup. Sequences are denoted by GenBank ID (reference strains in blue) or isolate ID. Roman numerals indicate genotype; numbers at the nodes indicate bootstrap values.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. All positions containing gaps and missing data were eliminated. There were a total of 437 positions in the final dataset. Sequences from the Netherlands and the United Kingdom are from [4].

**Table**

Characteristics of notified hepatitis A cases, Berlin, 14 November 2016–20 January 2017 (n=38)

| | MSM | Others[a] | Total |
|---|---|---|---|
| Number | 30 | 8 | 38 |
| Male patients | 30 of 30 | 7 of 8 | 37 of 38 |
| Median age (range) in years | 31 (24–52) | 34 (11–50) | 31 (11–52) |
| Hospitalised | 6 of 30 | 2 of 7[b] | 8 of 37[b] |
| Sexual contacts in non-household venues | 12 of 24[b] | NA | NA |
| Migration background | 13 of 25[b] | 1 of 4[b] | 14 of 29 |
| Drug use | 1 of 25[b] | NA | NA |

MSM: men who have sex with men; NA: not applicable.

[a] This category includes one heterosexual patient, one homosexual female patient and six male patients with unknown status.

[b] Information missing for some patients.

reinforce offering (i) vaccination to people with sexual behaviour at high-risk for HAV infection [10], and (ii) post-exposure prophylaxis to exposed contacts (active and passive immunisation is effective if administered within two weeks after exposure) [11].

This information was sent to practitioners who focus on treating HIV patients in Berlin, as well as to gay-oriented magazines, newsletters, webpages and specialised healthcare organisations. Furthermore, information was published in the weekly newsletter of the SOHSA and the Epidemiological Bulletin of the RKI [12].

## Discussion

We report on a recent increase of notified hepatitis A cases in Berlin, attributable to cases in MSM. The age distribution of MSM is comparable to that of MSM in previously described hepatitis A outbreaks [7,13]. The vast majority of cases was not vaccinated against hepatitis A indicating a need for targeted risk communication and vaccination campaigns. Of note, condom use is not a safeguard against HAV infection because it does not block the faecal-oral transmission route.

Interestingly, two different HAV sequences detected in cases from Berlin were recently identified in clusters of MSM in the Netherlands and in the UK [7]. The third sequence was identified in a cluster of six MSM cases in Munich and Frankfurt from August through October (data not shown). It is unclear why three different HAV strains concurrently circulate in Berlin among MSM. Apparently, Berlin's MSM scene is nationally and internationally well connected allowing for 'importation' and 'exportation' of HAV strains from or to various places in Europe.

For hepatitis A, the German electronic notification system does not capture sexual orientation. Thus, the magnitude of sexually transmitted hepatitis A is likely underestimated. The outbreak described here highlights the interconnectedness of MSM in Europe and the need to increase coverage of hepatitis A vaccination in this group.


### Acknowledgements

We would like to thank colleagues from the local public health authorities for collecting case data and organising to forward blood and stool specimens for strain typing. We also would like to acknowledge the following colleagues who reported to EPIS on possibly linked cases in other countries: Luise Müller (Denmark), Solveig Jore (Norway), Franz Allerberger (Austria), Lelia Thornton (Ireland), Harry Vennema (The Netherlands) and Lena Sundqvist (Sweden).

This work was supported by the Robert Koch-Institute and the German Federal Ministry of Health [grant number 1369-386 to J. Wenzel].


### Conflict of interest

None declared.

### Authors' contributions

DW JB and DSa have conducted enhanced surveillance of hepatitis A in Berlin, DW has written the manuscript MH and DSi have discovered the link of recent hepatitis A cases and MSM in Berlin Mitte and provided detailed case information. KM has monitored the situation at the federal level and communicated with European colleagues via EPIS. AB has investigated the cluster in Munich with links to Frankfurt, JW conducted the sequencing of hepatitis A viruses in this outbreak. MF has conducted the phylogenetic analysis and co-written the manuscript. All authors have revised the manuscript.

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# Outbreak of hepatitis A associated with men who have sex with men (MSM), England, July 2016 to January 2017

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**Citation style for this article:**
Beebeejaun K, Degala S, Balogun K, Simms I, Woodhall SC, Heinsbroek E, Crook PD, Kar-Purkayastha I, Treacy J, Wedgwood K, Jordan K, Mandal S, Ngui SL, Edelstein M. Outbreak of hepatitis A associated with men who have sex with men (MSM), England, July 2016 to January 2017. Euro Surveill. 2017;22(5):pii=30454. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.5.30454

Article submitted on 27 January 2017 / accepted on 02 February 2017 / published on 02 February 2017

**Between July 2016 and January 2017, 37 confirmed cases of hepatitis A with two unique IA genotype strains primarily among men who have sex with men, were reported across eight areas in England and Northern Ireland. Epidemiological and laboratory investigations indicate that these strains may have been imported several times from Spain, with secondary sexual transmission in the United Kingdom. Local and national public health services are collaborating to control this ongoing outbreak.**

Infection with the hepatitis A virus (HAV) is most commonly acquired through ingestion of contaminated food and water, and through faeco-oral contact. In the United Kingdom (UK) hepatitis A is a rare and mainly travel-associated disease, preventable by vaccination [1,2]. Sexually transmitted hepatitis A outbreaks among men who have sex with men (MSM) are well documented [3-6]. We describe an ongoing outbreak in the UK, primarily affecting MSM, caused by two concurrently circulating HAV strains previously not seen in the UK, as well as the intervention strategies that have been instigated to control the outbreak. Cases with the identical strains have been reported in other European countries, prompting the European Centre for Disease Prevention and Control (ECDC) to issue a rapid risk assessment in December 2016 [7].

## Case definition

A confirmed case was defined as a laboratory-confirmed HAV infection with the specific outbreak sequence of either VRD_521_2016 Strain 1 (Event 1) or RIVM-HAV16–090 Strain 2 (Event 2) and symptom onset after 31 June 2016 [7]. A probable case was defined as a laboratory-confirmed HAV infection (not yet sequenced) with symptom onset after 31 June 2016, with contact with a confirmed case and/or who identifies as MSM.

## Outbreak description

Between July 2016 and January 2017, 37 confirmed cases with either strain 1 or 2 were detected across England as well as Northern Ireland (Figure 1), of which 28 identified as MSM. Of the 37 cases, 24 were Strain 1 and 13 were Strain 2. In addition, 15 probable cases (all MSM), primarily in London, were identified, and typing results are awaited.

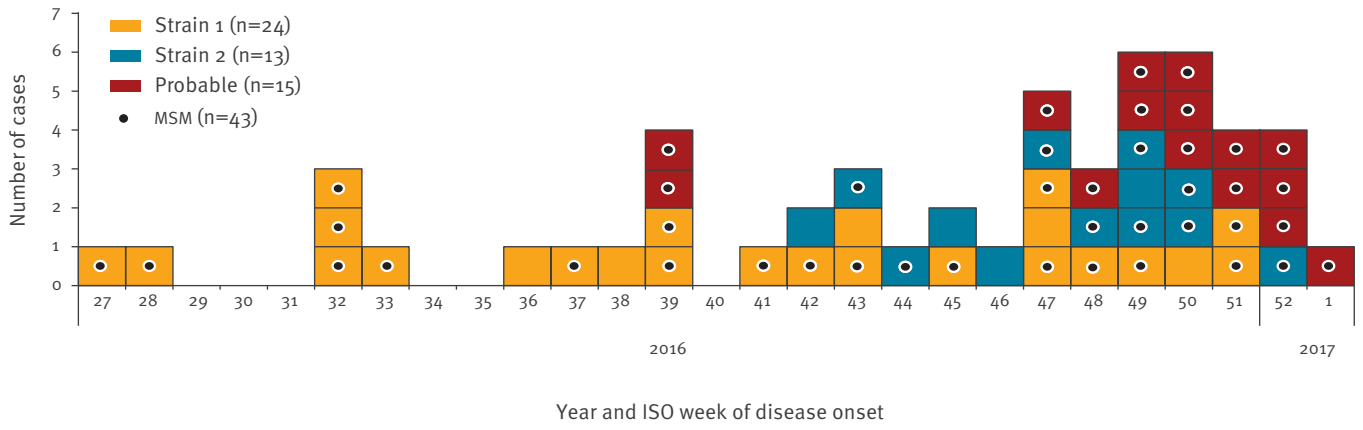
Strain 1 was first identified by the Virus Reference Department, Public Health England, London, in July 2016. The sequence had not been seen previously in the UK and phylogenetic analysis (Figure 2) showed a clear relation to sequences derived from travellers returning from Central and South America.

Strain 1 cases were reported in eight geographically distinct areas in England and Northern Ireland (Figure 3).

Of 24 Strain 1 cases, 22 were male, median age 35 years (19–63 years), 19 identified as MSM and eight reported travel within the incubation period, seven of which to Spain (Table).

**Figure 1**

Probable and confirmed cases of hepatitis A among men who have sex with men, England and Northern Ireland, July 2016–January 2017 (n=52)




ISO: International Organization for Standardization; MSM: men who have sex with men.

Strain 2 was first notified through the European Union Early Warning and Response System (EWRS) message from the Netherlands in October 2016 related to two MSM cases at EuroPride 2016, which took place in Amsterdam in July/August 2016. This genotype sequence was detected in 13 cases across six regions in England between November 2016 and January 2017 (Figure 3). Of the 13 cases, 12 were male, median age 39 years (range: 29–78), nine identified as MSM and 11 travelled during the incubation period, of which seven to Spain and two to Germany (Table). Of note, Strain 2 has mainly been reported in MSM in London to date. Characteristics of concern among cases were noted, including infection in a sex worker with multiple partners, co-infection with sexually transmitted infections (STIs) and use of sex-on-site premises and apps (Grindr, Recon) (Table).

## Control measures

Public Health England (PHE) declared a national incident in December 2016. Local and national laboratory, epidemiology and health protection teams contributed to the response, which comprised: (i) enhanced surveillance for MSM-associated cases through an adapted questionnaire [8], (ii) a joint letter with the British Association for Sexual Health and HIV (BASHH) to all members alerting them to the outbreak and recommending vaccination of at-risk MSM in outbreak areas, according to national guidelines [9,10], testing cases for other STIs and partner notification, (iii) disease information and targeted hygiene advice to the public through the National Health Service web portal [11], (iv) liaising with lesbian, gay, bisexual, and transgender (LGBT) and sexual health charities, gay-dating apps and gay venues to raise awareness through social media and health promotion visuals, and (v) giving post-exposure prophylaxis to household and sexual contacts.

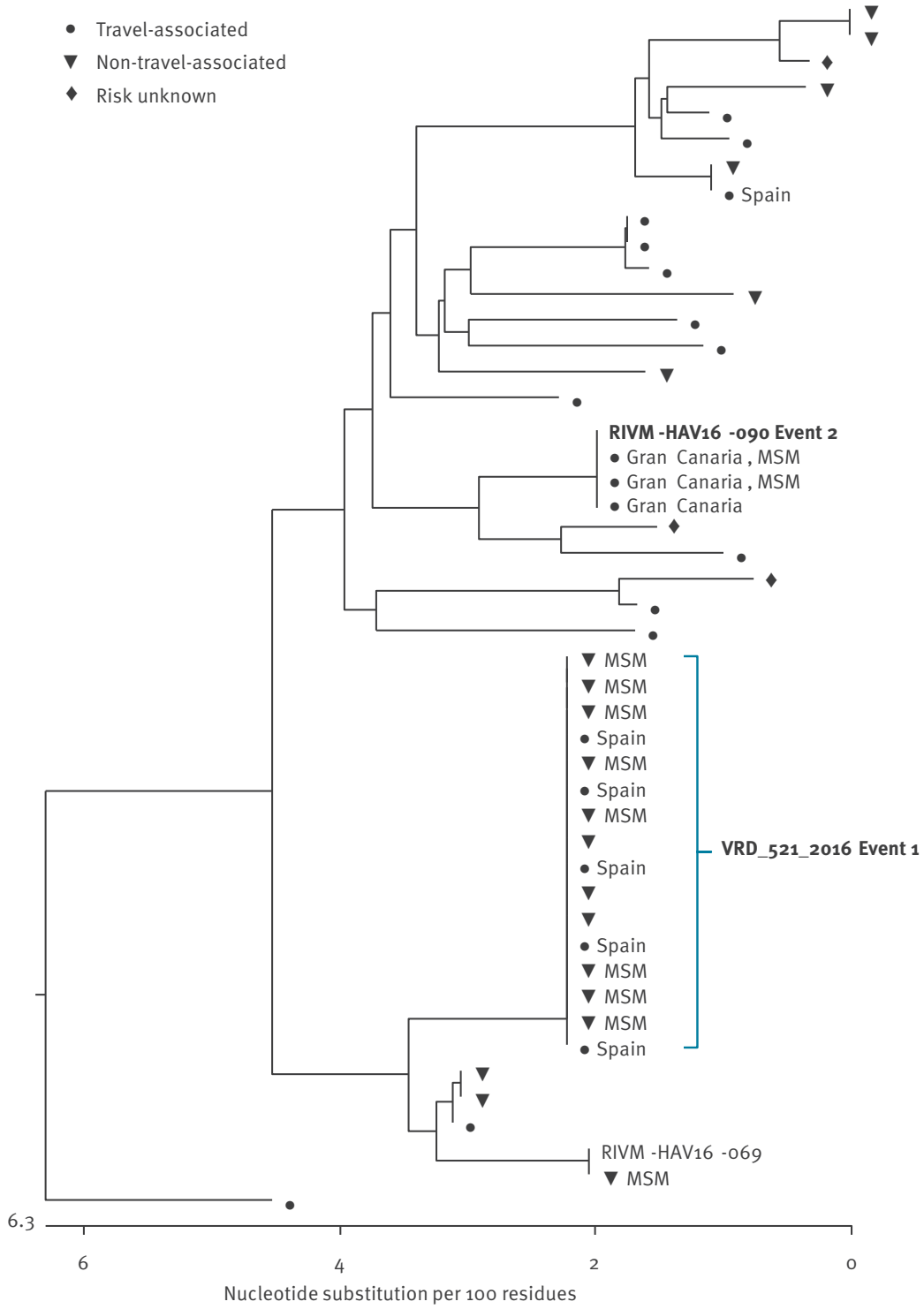
## Discussion

As at 24 January 2017, 37 HAV infections with two sequences have been identified in eight UK areas, mostly among MSM (median age: 35 years; range: 19-56). HAV infection is most commonly acquired through contaminated food or water. In this outbreak however, epidemiological and laboratory investigations suggest multiple importations from several regions of Spain with secondary sexual transmission within the MSM population in the UK, as nine of the confirmed MSM cases reported travelling to Spain during the incubation period. Ireland, Sweden, Luxembourg and Germany have reported hepatitis A cases with identical viral sequences, some with history of travel to Spain during the incubation period. Spain has reported an increase in male HAV infections, but no further details were available [7]. This outbreak highlights the key role sequencing can play in outbreak detection, as well as the added value of a common European platform to share epidemiological and virological information.

While the two concurrently circulating strains are virologically distinct, the public health response is intended to address both. Although it has not been possible to establish epidemiological links between all cases within geographical clusters, it is likely that cases are related either through undisclosed sexual contacts or other routes since neither strain is commonly circulating in England. These missing epidemiological links are not unexpected when trying to capture sexual history via short questionnaires, particularly since some cases reported anonymous sex with multiple partners. However, the questionnaires revealed sex-on-premises venues (saunas, clubs) and social networking (dating apps) as potential drivers of the outbreak. While these findings can help focus interventions, they are of particular concern in areas with

**Figure 2**

Phylogenetic analysis of virus strains from hepatitis A cases in England and Northern Ireland, July 2016–December 2016




HAV: hepatitis A virus; MSM: men who have sex with men.

The tree was constructed in MegAlign (DNAstar) using Clustal Key.

## Figure 3

Geographical distribution of hepatitis A cases among men who have sex with men, England and Northern Ireland, July 2016–January 2017 (n=52)

## Table

Characteristics of hepatitis A cases associated with the outbreak, England and Northern Ireland, July 2016–January 2017 (n=52)

| Region | Case status (strain) | Cases (n) | Median age (years) | MSM (n) | Spain | Notable characteristics |
|---|---|---|---|---|---|---|
| East Midlands | Confirmed (Strain 1) | 9 | 28 | 6 | 2 | One cluster of three cases of Strain 1 transmitted in a factory through environmental exposure. |
| | Confirmed (Strain 2) | 3 | 55 | 2 | 2 | |
| | Probable | 0 | NA | 0 | 0 | |
| | **Total** | **12** | **36** | **8** | **4** | |
| South West | Confirmed (Strain 1) | 4 | 45 | 3 | 1 | One case operated a private meeting place, used by contacts and multiple anonymous men. |
| | Confirmed (Strain 2) | 1 | NA | 0 | 1 | |
| | Probable | 0 | NA | 0 | 0 | |
| | **Total** | **5** | **46** | **3** | **2** | |
| Hampshire | Confirmed (Strain 1) | 3 | 35 | 3 | 1 | Probable case is index case in this area. This case was diagnosed in Spain but never sequenced. Further spread through household and sexual contacts. |
| | Confirmed (Strain 2) | 0 | NA | 0 | 0 | |
| | Probable | 1 | NA | 1 | 1 | |
| | **Total** | **4** | **32** | **4** | **2** | |
| North East | Confirmed (Strain 1) | 3 | 41 | 3 | 1 | First identified case with likely importation from Spain. Further spread to two cases through household and sexual transmission. |
| | Confirmed (Strain 2) | 0 | NA | 0 | 0 | |
| | Probable | 0 | NA | 0 | 0 | |
| | **Total** | **3** | **41** | **3** | **1** | |
| London | Confirmed (Strain 1) | 2 | 31 | 2 | 0 | One Strain 1 case was a sex worker with multiple sexually-transmitted co-infections who reported sex in several gay saunas in London. Three cases reported using apps and websites to meet partners. One Strain 2 case reported 20 sexual contacts within the eight weeks prior to disease onset. |
| | Confirmed (Strain 2) | 6 | 35 | 4 | 3 | |
| | Probable | 12 | 34 | 12 | 1 | |
| | **Total** | **20** | **32** | **18** | **4** | |
| Yorkshire and Humber | Confirmed (Strain 1) | 1 | NA | 0 | 0 | All but one case reported travel; three to Spain and to Germany. One Strain 2 case reported sexual contact with multiple partners at a gay sauna in London. |
| | Confirmed (Strain 2) | 1 | NA | 1 | 1 | |
| | Probable | 1 | NA | 1 | 0 | |
| | **Total** | **3** | **NA** | **2** | **1** | |
| North West | Confirmed (Strain 1) | 0 | NA | 0 | 0 | |
| | Confirmed (Strain 2) | 1 | NA | 1 | 0 | |
| | Probable | 1 | NA | 1 | 0 | |
| | **Total** | **2** | **43** | **2** | **0** | |
| East of England | Confirmed (Strain 1) | 0 | NA | 0 | 0 | |
| | Confirmed (Strain 2) | 1 | NA | 1 | 0 | |
| | Probable | 0 | NA | 0 | 0 | |
| | **Total** | **1** | **NA** | **1** | **0** | |
| South Midlands | Confirmed (Strain 1) | 1 | NA | 1 | 1 | |
| | Confirmed (Strain 2) | 0 | NA | 0 | 0 | |
| | Probable | 0 | NA | 0 | 0 | |
| | **Total** | **1** | **NA** | **1** | **1** | |
| Belfast | Confirmed (Strain 1) | 1 | NA | 1 | 1 | |
| | Confirmed (Strain 2) | 0 | NA | 0 | 0 | |
| | Probable | 0 | NA | 0 | 0 | |
| | **Total** | **1** | **NA** | **1** | **1** | |
| **Grand total** | | **52** | **36** | **43** | **16** | |

MSM: men who have sex with men; NA: not applicable.

large, active MSM populations, such as London, where several of the recent cases have been reported.

This outbreak also highlights the need for HAV awareness among MSM and sexual health professionals and the need for health promotion materials that focus on both infection and vaccination. Innovative and evaluated communication strategies with targeted messaging through social media, apps and venues also need to be readily available to public health agencies.

Hepatitis A vaccination for MSM in England is currently a risk-based recommendation [9,10]. For the purpose of this investigation, the vaccination status of the cases was not included in the analysis. While some may advocate for a universal MSM vaccination policy, it may not be cost-effective or affordable for local governments who commission sexual health services. Vaccine availability also needs to be taken into account as it may impact the ability to vaccinate a large number of individuals in a short timeframe. Enhanced surveillance for HAV in MSM will allow monitoring of the evolving outbreak as well as evaluating intervention impact, and gain a better understanding of HAV transmission in this population.


## Acknowledgements

We would like to thank all the regional PHE health protection staff involved in the outbreak investigation and management to date.


## Conflict of interest

None declared.

## Authors' contributions

All the authors contributed to the outbreak investigations described here, the presentation of information and the final drafting of the manuscript.

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# Swine influenza A (H1N1) virus (SIV) infection requiring extracorporeal life support in an immunocompetent adult patient with indirect exposure to pigs, Italy, October 2016

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**Citation style for this article:**
Rovida F, Piralla A, Marzani FC, Moreno A, Campanini G, Mojoli F, Pozzi M, Girello A, Chiapponi C, Vezzoli F, Prati P, Percivalle E, Pavan A, Gramegna M, Iotti GA, Baldanti F. Swine influenza A (H1N1) virus (SIV) infection requiring extracorporeal life support in an immunocompetent adult patient with indirect exposure to pigs, Italy, October 2016. Euro Surveill. 2017;22(5):pii=30456. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.5.30456

Article submitted on 17 January 2017 / accepted on 02 February 2017 / published on 02 February 2017

**We describe a case of severe swine influenza A(H1N1) virus infection in an immunocompetent middle-aged man in October 2016 in Italy who had only indirect exposure to pigs. The patient developed a severe acute distress respiratory syndrome which was successfully supported by extracorporeal membrane oxygenation and treated with antiviral therapy. The sole risk factor for influenza was a body mass index › 30 kg/m². After a month of hospitalisation, the patient was discharged in good health.**

## Case description

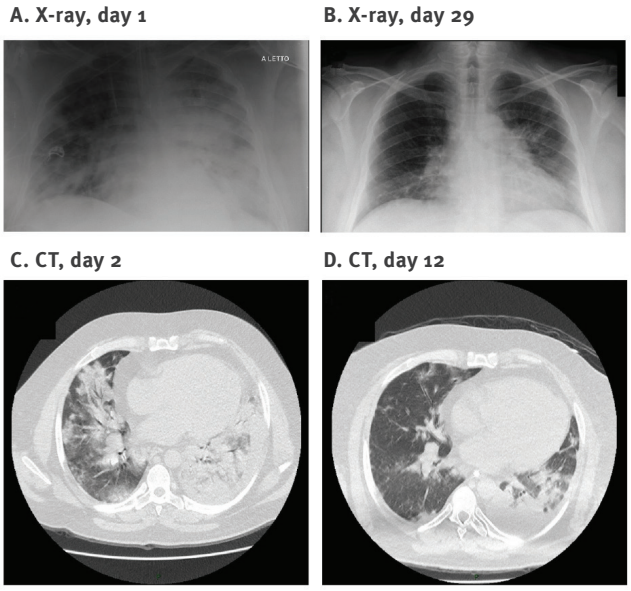
In early October 2016, a man in his 40s with underlying obesity (body mass index › 30 kg/m2) presented at the emergency department of our hospital after four days of rhinitis, cough, fever and dyspnoea. The patient was hospitalised due to hypoxaemia (PaO2/FIO2 = 190), hypocapnia, hyperlactataemia (3.6 mmol/L), dyspnoea and bilateral interstitial pneumonia, as shown by chest X-ray and computed tomography (CT) (Figure 1).

On the following day, the patient's clinical condition worsened (PaO2/FIO2 = 65) and he was transferred to the intensive care unit (ICU) with severe acute distress respiratory syndrome (ARDS). The CT scan at ICU admission is shown in Figure 1. The patient was first supported with helmet continuous positive airways pressure (CPAP) and then with invasive mechanical ventilation. On the same day, a nasal swab sample and a bronchoalveolar lavage (BAL) were collected and tested by real-time RT-PCR and PCR for a panel of 12 respiratory viruses [1,2]. Influenza A virus was detected, with high viral load in the BAL (9.7 × 107 RNA copies/mL) and a lower viral load in the nasal swab (4.5 × 102 RNA copies/mL), while the results for the remaining 11 viruses were negative. The BAL was negative for the most common bacteria and fungi by standard cultures. Oseltamivir treatment (75 mg twice a day) was started.

Three days after admission, low-flow veno-venous extracorporeal membrane oxygenation (ECMO) was initiated in order to allow hyperprotective mechanical ventilation with low tidal volume (‹ 4 mL/kg ideal body weight, which was calculated to be 65 kg). Eight days after admission, respiratory conditions improved and the patient was disconnected from ECMO. The influenza A virus RNA load had decreased considerably (2.0 × 104 copies/mL in BAL and 0 RNA copies/mL in the nasal swab). Five days later, the BAL was negative for influenza A virus RNA but the patient experienced a super-infection with meticillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and therapy with linezolid and piperacillin/tazobactam was administrated. After disconnection from ECMO support, the

**Figure 1**

Chest X-ray and computed tomography in a patient with severe swine influenza A(H1N1), Italy, October 2016




CT: computed tomography.

The X-ray taken on admission (A) showed bilateral opacities. A follow-up chest X-ray performed on day 29 showed almost complete regression of the opacities (B). Chest CT on day 2 after admission (C) showed extensive bilateral consolidation with alveolar parenchymal consolidations and ground-glass opacity (right end). CT on day 12 after admission showed partial regression of consolidation in the right median-lower lobe with residual little areas of ground-glass opacity and improved ventilation of the lower left lobe (D).

patient was gradually weaned from mechanical ventilation and subsequently from CPAP.

After 16 days of ICU, as the patient's clinical condition was improving, he was transferred to the pneumology ward and after 30 days of overall hospital stay discharged in good health.

## Virological findings

Molecular subtyping of the influenza A strain (A/Pavia/65/2016) was unsuccessful using real-time RT-PCRs specific for human influenza subtypes H1 and H3 directly on biological samples. On day 7 of admission, partial nucleotide sequences of the nucleoprotein and non-structural genes, obtained in an RT-PCR that amplifies all eight segments of the influenza A genome [3], showed that the A/Pavia/65/2016 strain was an influenza A(H1N1) virus of swine origin. The A/Pavia/65/2016 strain was propagated in embryonated specific pathogen–free chicken eggs using BAL and swab samples as inoculum and all eight genome segments were sequenced using the MiSeq platform (Illumina, San Diego, US) as previously described [4] (GenBank accession numbers KY368147–154). The data were de novo assembled on BaseSpace Cloud (Illumina, San Diego, US) with the DNAStar application and analysed with the Lasergene package software (version 10.1.2).

Phylogenetic analyses were performed using MEGA6 software [5]. A phylogenetic tree of the haemagglutinin (HA) and neuroaminidase (NA) genes confirmed that the A/Pavia/65/2016 strain was closely related to the European avian-like swine influenza A(H1N1) virus (Figure 2). The phylogenetic analysis of the internal genes excluded genome reassortments and showed that all eight segments derived from the Eurasian avian-like lineage.

The patient had not had any direct contact with pigs but lived with a brother employed as a breeder on a pig farm. When interviewed, the patient's brother reported having had mild respiratory symptoms, in late September to early October. Virological investigations on the pig farm mid-October showed an absence of clinical signs in the animals, but nasal swabs collected from twelve weaning pigs resulted positive by PCR for influenza A, although at a low viral load. Partial genome sequencing of one of the strains (A/swine/Italy/285919/3/2016) included complete HA, NA, M, NS genes and partial PB2, PB1, PA and NP segments (GenBank accession numbers KY368155–162) and proved its close relationship with the A/Pavia/65/2016 strain (Figure 2 and Table), placing it among the Eurasian swine influenza virus (SIV) avian-like strains circulating in Italy.

Genetic distance analysis showed that the A/Pavia/65/2016 strain shared 98.6–100.0% nucleotide identity with the A/swine/Italy/285919/3/2016 strain (Table) and 97.3–99.6% with the Eurasian SIV strain circulating in Italy during 2010, while a lower nucleotide identity (91.1–94.7%) was observed with the SIV strain (A/Netherlands/3315/2016 H1N1) recently detected in an ICU patient in the Netherlands [6].

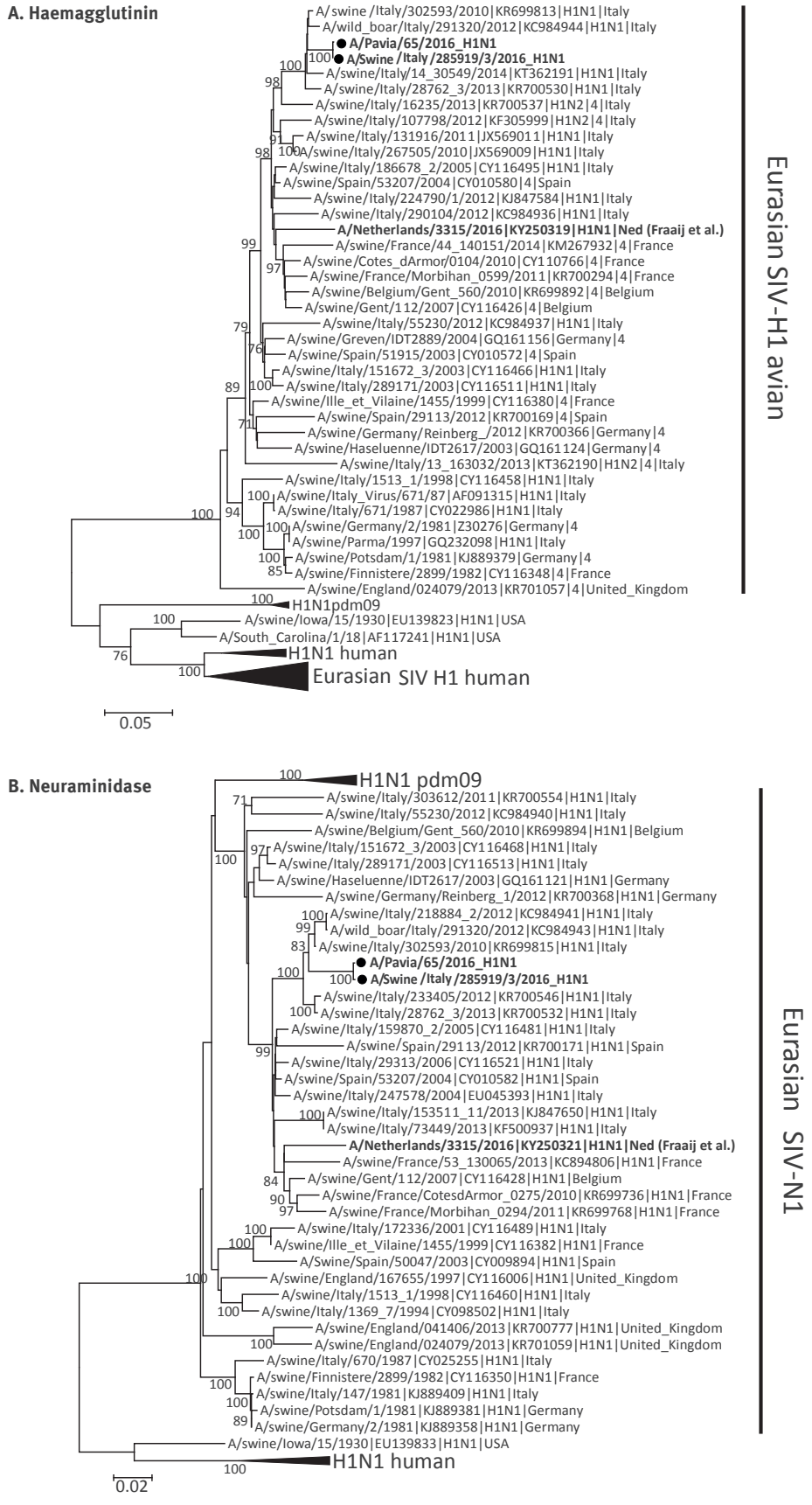
To confirm the indirect exposure of the patient to the swine influenza A strain, a serum sample of the patient's brother was tested by haemagglutination-inhibition (HI) test [7]. To remove potential nonspecific inhibitors, human serum was heat-inactivated, adsorbed with chicken red blood cells and treated with receptor-destroying enzyme. The HI assay was performed using the A/Pavia/65/2016 and two reference SIV: A/swine/CA/3633/84 H3N2 and A/swine/Finistere/2899/82. Two-fold serum dilutions were tested starting at 1:10 and showed in the serum sample of the patient's brother higher antibody titres against the A/Pavia/65/2016 and H3N2 (A/swine/CA/3633/84) strains (1:320) than against the H1N1 (A/swine/Finistere/2899/82) strain (1:20).

## Background

Since the first isolation of a SIV from a human in 1974 [8], sporadic human cases of SIV have been reported in the United States, Canada, Europe and Asia [9-11]. Although most cases of SIV in humans are associated

## Figure 2

Phylogenetic relationship of human and swine influenza A(H1N1) virus strains based on complete haemagglutinin and neuraminidase nucleotide sequences, compared with patient isolate, Italy, October 2016




SIV: swine influenza virus.

The unrooted trees were created by neighbour-joining method implemented in the MEGA 6.0 software and bootstrapped with 1,000 replicates. The pairwise distances were computed using the Kimura 2-parameter model. Only bootstrap values higher than 70% are shown. Patient and pig haemagglutinin and neuraminidase sequences from our study are marked with a black circle and bold letters. Scale bars indicate nucleotide substitutions per site. Sequence reported in Fraaij et al. [6] are highlighted in bold. The other sequences used in the phylogenetic trees were obtained from Watson et al. [12] as well as from the GenBank database.

**Table**

Nucleotide identity of the eight genome segments of the influenza A/Pavia/65/2016 from the patient and the influenza A/swine/Italy/285919/3/2016 (Sw16) obtained on the pig farm vs reference strains, Italy, October 2016

| Gene | Influenza A(H1N1) strain | | | | |
|---|---|---|---|---|---|
| | Sw16 | Sw10 | SwNed16 | Nc99 | Ca09 |
| PB2 | 99.9[a] | 98.3 | 91.2 | 81.7 | 83.5 |
| PB1 | 99.8[a] | 98.2 | 92.7 | 80.6 | 86.3 |
| PA | 99.8[a] | 98.1 | 94.0 | 80.6 | 81.4 |
| HA | 99.9 | 97.7 | 91.1 | 67.3 | 66.0 |
| NP | 98.6[a] | 98.5 | 94.7 | 78.8 | 80.4 |
| NA | 99.9 | 97.3 | 91.8 | 74.2 | 88.5 |
| M | 100.0 | 92.6 | 93.9 | 83.0 | 92.0 |
| NS | 100.0 | 99.6 | 91.8 | 81.8 | 76.5 |

Sw16: Eurasian swine influenza virus A/swine/Italy/285919/3/2016; Sw10: Eurasian swine influenza virus A/swine/Italy/302593/2010 (H1N1); SwNed16: severe swine influenza A(H1N1) case A/Netherlands/3315/2016(H1N1) detected by Fraaij et al. [11]; Nc99: human influenza virus A/New Caledonia/20/1999 (H1N1); Ca99: human influenza virus A/California/07/2009 (H1N1pdm).

GenBank accession numbers represent sequences from the eight segments of influenza A virus: Sw16 (KY368155–162), Sw10 (KR699810–817), SwNed16 (KY250316–323), Nc99 (AY289929, CY033623–629); Ca09 (NC026431–438).

[a] Nucleotide comparisons were performed with partial sequences of four of the eight genomic segments (PB2: 1,372 nt; PB1: 455 nt; PA: 539 nt; NP: 247 nt).

with mild respiratory syndromes [8-11], a case of severe SIV has recently been reported in the Netherlands [6]. Exposure to pigs is often considered a risk factor for human SIV infections [9] and seroepidemiological studies have demonstrated increased rates of SIV infection in occupationally exposed humans [8-10]. People with exposure to swine may be the first to be infected in the event of a novel virus becoming epizootic in a swine herd, and those who work with swine may operate as a bridge for transmission of the virus to their communities [9].

## Discussion

Exposure to swine is often considered a risk factor for human SIV infections [8]. Here we describe a severe case of swine influenza A(H1N1) virus infection requiring ECMO in an adult immunocompetent man with a BMI › 30 as a risk factor, who had indirect exposure to pigs through a brother working as a breeder on a pig farm. Twelve pigs of the farm tested positive for influenza A, and the strain sequenced from one of them was closely related to the virus recovered from the patient. In addition, antibodies against swine influenza A strains (including the strain recovered from the patient) were detected in the serum sample of the patient's brother. These data support the hypothesis that SIV infecting our patient was circulating on the pig farm and the patient's brother might have operated as a bridge for transmission of the virus.

As SIV infection in humans is mild in most cases, its frequency might be underdiagnosed [9,11]. Nevertheless, in the same month of October, Fraaij et al. reported a case of severe infection caused by swine influenza A(H1N1) in a child requiring ECMO support in the Netherlands [6]. In our phylogenetic analysis of HA and NA, this Dutch strain [6] and the strain A/Pavia/65/2016, circulating in the same period in Europe, clustered into distinct branches of the trees.

## Conclusion

We have reported here a case of severe swine influenza A infection following indirect exposure to pigs. One possible path of infection could be human-to-human. However, other routes (e.g. contact with contaminated clothing, surfaces etc.) cannot be excluded. These data further highlight the need of strict surveillance of influenza in humans and in animals.


### Acknowledgements

We thank all the technical staff for handling the specimens and performing the assays. We thank Dr Enrico Arioli for the supporting in veterinary investigations. We thank Mrs Daniela Sartori for manuscript editing. This work was sustained by Ministero della Salute, Ricerca Corrente grant no. 80206 to Dr. Elena Percivalle; Ministero della Salute, Ricerca Corrente grant no. 80622 to Dr. Antonella Sarasini; Ministero della Salute, Research project PRC2015006 –"Epidemiology of Influenza virus type A, B, C, D in Italian swine and bovine herds and their possible reverse zoonotic aspects".


### Conflict of interest

None declared.

### Authors' contributions

Wrote the manuscript: FR, AP, FCM, AM, FB; managed the patient: FCM, FM, MP,GAI; performed laboratory investigations: GC, AG, EP, AM, CC, FV, PP; revised the manuscript: FR, AP, FB, FCM, AM; coordinated the study: FB, GAI, AM, FCM, AP, GC, AP, MG.

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# Emergence of the lymphogranuloma venereum L2c genovariant, Hungary, 2012 to 2016

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**Citation style for this article:**
Petrovay F, Balla E, Erdősi T. Emergence of the lymphogranuloma venereum L2c genovariant, Hungary, 2012 to 2016 . Euro Surveill. 2017;22(5):pii=30455. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.5.30455

Article submitted on 26 September 2016 / accepted on 20 January 2017 / published on 02 February 2017

**In eastern Europe, few countries have so far reported laboratory-confirmed cases of lymphogranuloma venereum (LGV). Here we describe 22 LGV cases in men who have sex with men (MSM) detected in Hungary from November 2012 to July 2016. Sequence analyses show that 16 of these 22 cases were affected by the L2c genovariant, with from 2012 to 2014, one LGV L2c case detected per year, followed by seven cases in 2015 and six up to July 2016. Of the 16 total L2c LGV cases, 10 had severe haemorrhagic proctitis. These findings are concerning as cases with this new genovariant among MSM have not been frequently reported in Europe to date. More research is needed to assess the spread of the L2c genovariant and its potential association with virulence and severe clinical manifestation.**

## Introduction

Among men having sex with men (MSM), lymphogranuloma venereum (LGV) causing proctitis and anorectal ulceration has been a re-emerging sexually transmitted disease in Europe since 2003, and many western and northern European countries have reported cases regularly [1,2]. In southern and eastern Europe however, only a few cases have been documented so far, the first in the Czech Republic in 2010, followed by Hungary in 2012 and Slovenia in 2015 [3-5]. In the Czech Republic, the number of detected cases has steadily increased since the initial identification [6].

The current LGV epidemic in western Europe is caused by the L2 biovar of *Chlamydia trachomatis* with the predominance of the L2b genovariant [7,8]. While most infections have occurred anorectally in human immunodeficiency virus (HIV)-positive MSM who have a high-risk behaviour, only few urethral infections have been reported [9]. Moreover a new LGV genovariant, designated L2c, which is a recombinant of L2 and D strains, has also been identified, but only a few persons affected by it have been documented so far [5,10].

The L2c genovariant was originally isolated from a case with severe haemorrhagic proctitis, showing a unique clinical pattern, and was described as a hypervirulent LGV strain developing cytotoxic phenotype in culture [10]. Despite this, there is no evidence in the literature confirming the association of this genovariant with more severe clinical manifestations. So far, the L2c genovariant is relatively rarely reported and only its emergence is mentioned in the updated European guideline on the management of LGV [11]. There are moreover no epidemiological data on its prevalence in western European countries.

In eastern Europe testing for LGV has only recently been implemented so detection of cases has just begun. There are no published data about the LGV genotypes identified in the Czech Republic and only one confirmed L2c genotype was reported from Slovenia in 2015 [3,5,6]. In another neighbouring country, Austria, a high rate of non L2b variants among reported LGV cases (7/15) was observed in 2008, however sequenced-based identification was not performed at that time [12].

In this report we present findings of laboratory-confirmed LGV cases in Hungary from November 2012 through June 2016 as well as the sequence analysis results of isolates collected.

## Methods

### Clinical specimens

Since November 2012, i.e. after the first laboratory-confirmed LGV case in Hungary [4], 21 further cases have been investigated and confirmed as LGV in the Bacterial STI Reference Laboratory of the National Centre for Epidemiology, Budapest. Swab samples from the anus, urethra, a penile ulcer or an inguinal bubo as well as native blood samples were taken by venereologists of various genitourinary medicine

(GUM) clinics and sexually transmitted infection (STI) outpatient wards in Budapest. While in the capital, these clinics and wards offer their services to patients from the whole country. The physicians provided clinical information based on the patients' statements and on the respective physical examinations. Medical history (HIV serostatus) was also included.

### Laboratory investigations

LGV cases were laboratory-confirmed in a two-step protocol. Following DNA isolation, all samples were screened by an in-house *C. trachomatis* PCR targeting the plasmid gene. When the PCR was positive for *C. trachomatis* genetic material, LGV infection was confirmed with a *pmp*H real-time PCR which differentiates the L serovars from the other urogenital serovars of *C. trachomatis* [13]. The samples were then stored at −20 °C until further identification of the genovariants. A fragment of the *omp*1 gene (ca 1,070 bp) was subsequently PCR amplified using a previously described protocol [14]. In order to genotype, a partial sequence from this 1,070 bp amplicon (ca 900bp) was obtained by sequencing, and aligned to reference sequences from GenBank representing different LGV variants: L2a (GenBank accession number: AF30485); L2(AM884176); L2b(AM884177); L2c (NC_015744); L2d (EF460797); L2e (EF460798); L2f (EU676181); and L2g (EU676180).

Additionally all anogenital samples were screened by PCR for *Neisseria gonorrhoeae and Treponema pallidum*. *T. pallidum* serology was performed simultaneously.

## Results

### Patients' characteristics

LGV was confirmed in altogether 22 cases between 2012 and 2016. The distribution of the 22 LGV cases in time, after the first identified case in 2012, was as follows: two LGV in 2013, three in 2014, eight in 2015 and eight until July 2016 respectively.

The age distribution showed that the 25–34 years age group included most cases (10/22) followed by the 35–44 years age group with seven cases (Table).

Of the 22 cases, 13 came from Budapest or from the neighbouring cities of Pest county. The remainder were from various locations in the rest of Hungary. Nine cases mentioned having unprotected sexual contact with one or more foreign partners and/or visiting abroad recently. A total of 16 cases could not identify either their respective contact(s) or the possible site/time of infection. Only two cases were identified through contact tracing, one of them having serious proctitis, the other with an asymptomatic urethral carriage of the pathogen. The distribution of the 22 positive samples was as follows: 14 anal swabs, three urethral swabs, four penile swabs taken from the ulcerative lesions and one inguinal aspirate. Proctitis was the most common clinical manifestation observed among the cases (n=14). Other clinical symptoms included inguinal lymphadenopathy (n=6 cases), penile erosion (n=5), perianal ulceration (n=3), and urethral discharge (n=3). In two cases, patients suffering from rectal symptoms had been misdiagnosed and treated for irritable bowel disease (IBD) before the correct diagnosis of LGV was established.

### Laboratory findings

Sequence analysis of the LGV isolates showed that 16 of the sequenced samples (n = 21) were identical to L2c, three were identical to L2b, while two sequences could not be identified using the previously known reference sequences. These were designated L2bV1 according to a recent article [15]. The sequencing of one isolate failed in spite of repeated attempts because of low copy number of DNA. Distribution of cases with the L2c genovariant in time was as follows: one identified in 2012, in 2013 as well as in 2014; seven in 2015 and six until July 2016. Three patients with LGV infection caused by the L2c genovariant reported a recent visit to western European countries. Severe haemorrhagic proctitis was observed in 10 of the 16 LGV cases caused by the L2c genovariant. This particular manifestation was not seen in cases with the other genovariants.

Among the total 22 LGV cases, co-infection with *N. gonorrhoeae* was present in seven cases. *T. pallidum* DNA was detected in one case, however syphilis serology revealed that six patients suffered from a recent infection, nine had had previous syphilis infection, and only seven were seronegative. Concerning HIV status, 17 of 21 cases with this information available were HIV-positive (Table). Among severe proctitis cases (n=10), all of them affected by the L2c genovariant, co-infection rates were as follows: two patients' serological results suggested recent syphilis, one patient was co-infected with gonorrhea while another two patients showed both characteristics, i.e. possible co-infection with *N. gonorrhoeae* and *T. pallidum* (the latter was assumed by high reactive serological results).

## Discussion

As in the Czech Republic and countries of western Europe, we have noted an increasing number of LGV cases in Hungary after identification of the first case, which took place in November 2012. This increase may be due to the growing awareness of LGV among venereologists, which plays an important role in improved detection. Our STI laboratory is the only referral centre in Hungary that does confirmatory LGV testing and typing. Furthermore the institute's task as the National Centre for Epidemiology, also includes organising courses and giving lectures about the situation of the STI surveillance and the current LGV status in Hungary. These efforts have likely led to the increase of detected LGV cases.

There are no specialised MSM clinics in Hungary where routine LGV screening is offered to any patients. Symptomatic MSM patients usually attend GUM clinics

**Table**

Characteristics of 22 laboratory-confirmed lymphogranuloma venereum cases, Hungary, 2012–2016

| Characteristics | Sequenced genovariants[a] Number of cases | | | Total |
|---|---|---|---|---|
| | L2c | L2b | L2 variant[b] | |
| | 16 | 3 | 2 | 22[a] |
| **Age group (years)** | | | | |
| 15–24 | 3 | 0 | 0 | 3 |
| 25–34 | 8 | 1 | 1 | 10 |
| 35–44[a] | 4 | 2 | 0 | 7 |
| 45–54 | 1 | 0 | 1 | 2 |
| **Localisation of the LGV infection** | | | | |
| Rectum | 10 | 3 | 1 | 14 |
| Urethra[a] | 1 | 0 | 1 | 3 |
| Penile ulcer | 4 | 0 | 0 | 4 |
| Inguinal lymph node | 1 | 0 | 0 | 1 |
| HIV status[c] | | | | |
| Positive[a] | 11 | 3 | 2 | 17 |
| Negative | 4 | 0 | 0 | 4 |
| **Co-infection** | | | | |
| Gonorrhoea | 5 | 1 | 1 | 7 |
| Syphilis | 1 | 0 | 0 | 1 |
| **Reinfection** | | | | |
| Yes | 2 | 0 | 0 | 2 |
| No[a] | 14 | 3 | 2 | 20 |

HIV: human immunodeficiency virus; LGV: lymphogranuloma venereum.

[a] The genovariant could not be sequenced and identified in one case.

[b] These variants could not be identified using the previously known reference sequences. Their sequences were identical to a sequence with GenBank accession number JX971936.1 [17] and, according to a recent article [15], the variants were designated L2bV1.

[c] HIV-status was not available in one case.

or consult private venereologists or proctologists. They are not necessarily screened for LGV, however multiple site screening for bacterial STIs (chlamydia, gonorrhea, syphilis) is frequently performed. When LGV is diagnosed, the LGV status of asymptomatic partners can also be revealed in case of successful contact tracing. It is notable that a number of LGV cases in our study had visited abroad (e.g. Germany, Spain, the Netherlands) and had had foreign sexual partners besides multiple anonymous contacts and unprotected sexual practices. Difficulties in partner notification made it almost impossible to identify the actual sources of infection, whether in Budapest, the rest of Hungary or abroad.

Patient characteristics in our report were comparable to those reported by western European countries where LGV has been screened routinely for almost a decade, as well as to those in the recent report from the Czech Republic [6,11]. In our study, LGV was not frequent (n=3) in the youngest MSM age groups (≤24 years) and most patients (10/22) were 25–34 years of age. The majority (n=17) of LGV patients were HIV-positive. Moreover many (9/22) had a concomitant STI and syphilis serology showed reactivity in 14 patients. *C. trachomatis* was detected mainly (n=14) from the rectum of patients with proctitis. Interestingly however, in seven cases it was isolated from the urethra or a penile ulcer with symptoms of urethral discharge or ulceration, and in one case from an inguinal bubo (Table).

The relatively high rate of urethral/penile LGV positivity (7/22) among our LGV patients contrasts with data reported in the literature and may be explained by a great number of misdiagnosed rectal infections where patients were not tested for LGV at all [16]. One of the LGV positive urethral samples originated from an asymptomatic contact of a previously diagnosed rectal LGV patient. For this contact, the rectal sample had proved LGV negative, which points to the crucial roles of contact tracing, and testing for extra-anal regions as well. Clinically, penile ulcers may be misdiagnosed as primary syphilis so multiple testing for bacteria causing STIs including LGV is also essential in high risk MSMs. This allows detecting possible co-infections as well.

Based on the current European Guideline, anorectal *C. trachomatis* screening should be performed routinely in MSM reporting risky sexual behaviour [11]. In case of patients' symptomatic urethritis or ulcerative genital lesions the screening should be also extended to these extra-anal anatomic sites, as a rectal sample alone may be negative for LGV resulting in a misdiagnosis.

In contrast to western Europe, where the L2b genovariant has been reported to predominate, the sequence analysis in our study in Hungary revealed that the majority (n=16) of the LGV patients were caused by the recently described L2c genovariant. Conversely, the L2b genovariant was found in only three cases [7,11]. To our knowledge there is no report to date describing such a high rate of the L2c genovariant in Europe. In our patient population, all four HIV-negative patients had an LGV infection with the L2c genovariant and interestingly this genovariant was present in six of eight patients having extra-anal manifestations (Table).

These findings suggest that the new genovariant L2c of *C. trachomatis* LGV strains may have started to spread in Europe, hence continued analyses of sequences from further detected LGV cases are needed to confirm this. Genotyping is a useful tool for identifying outbreaks, confirming epidemiological links, revealing new variants and their role in certain groups of patients and in disease severity.

We found that half of the patients with severe L2c proctitis suffered from recent syphilis or from acute gonorrhea or both. These STI pathogens may contribute to the haemorrhagic symptoms and may cause more severe inflammation of the rectal mucosa.

It is noteworthy that two isolates in our study could not be identified as a formerly known L2 genovariant. Instead, their sequence analysis showed identity with an LGV variant sequence described in a Spanish study (GenBank accession number JX971936.1). This variant was presumably introduced to Europe through a South American-Spanish route and besides Spain it was also reported in France by Peuchant et al., where it was designated L2bV1 [15,17].

Our data have some limitations. Due to the high proportion of residents living in the capital area who represented more than half of the cases, our observations do not allow us to conclude as to the overall characteristics of all Hungarian patients affected by LGV.

Moreover, a number of LGV cases are likely to remain undetected at this time, as LGV is still underdiagnosed in Hungary, due to most clinicians being neither aware of the condition itself, nor of the targeted diagnostic possibilities. As part of our routine screening for multiple bacteria responsible for STIs of venereological patients, we tested all patients with clinically suspicious conditions for *C. trachomatis*, and when LGV was found among positive samples, this laboratory result was often unexpected for the clinicians. As LGV diagnostic tests are only available at our laboratory in Budapest, this may also affect the willingness to send samples from other cities for testing. In addition, many private venereologists or proctologists send anogenital samples of symptomatic MSM patients to other laboratories where only the presence of *C. trachomatis* DNA is revealed without further confirmatory typing. Hence the patients might receive treatment without having the correct diagnosis. In 2015, four LGV cases that had occurred earlier that year were diagnosed retrospectively with LGV, when *C. trachomatis* DNA samples were sent to our institute from another laboratory after clinicians contacted us for further investigation. These LGV cases would have surely remained otherwise unnoticed. Moreover two patients had been misdiagnosed and treated for IBD before the correct diagnosis of LGV was established. Therefore informing and educating gastroenterologists, especially proctologists about this condition in the MSM population would also be essential.

Another limitation of this study is that we do not have any precise data regarding the actual number of chlamydial infections in the country and thus the LGV rate among detected chlamydial infections could not be derived.

Finally, the lack of anamnestic data has to be mentioned, as only six patients provided information regarding their possible contacts or infections sites. This makes it extremely difficult to evaluate the epidemiological situation and to assess the source of L2c genovariants.

## Conclusion

LGV among MSM has already been spreading in Hungary since 2012 and based on Czech and Slovenian reports it is likely present in other southern or eastern European countries as well. Therefore intensified testing should be considered and increasing awareness among clinicians should be actively promoted. Venereologists and proctologists in particular should be informed of the presence of LGV in the MSM population, as well as on the typical symptoms, so that they can recognise the condition and be involved in a more effective, targeted surveillance system.

Public health interventions are needed to inform the MSM population about LGV by online channels or by social/health services. Our laboratory took part in an anonymous STI screening campaign including LGV that was performed in 2015 for 500 MSM persons (results are being processed). These screening efforts may result in a growing number of identified cases, a better understanding of the prevalence of different genovars and less misdiagnosis and wrong treatment of the infection. The virtual dominance of the L2c genovariant among Hungarian LGV cases warrants this variant to be further investigated in Europe.


### Acknowledgements

The authors would like to thank all venereologists of the various GUM clinics and the STI outpatient wards who provided the clinical samples for this study. Listed below chronologically:

Katalin Együd in Nyíregyháza, András Bánvölgyi, Márta Marschalkó, Viktória Várkonyi, Viktória Karászi, Péter Bognár, Béla Tóth, Anna Szegedi, Éva Csatár, György Lohinai, Ágnes Bihari, Margit Simola, and Béla Tamási in Budapest.

In particular we would like to thank Márta Benczik (Budapest) for her kind collaboration, Andrea Balázs and Judit Henczkó molecular biologists (National Centre for Epidemiology, Budapest) for their excellent technical input, and Naraporn Somboonna (Chulalongkorn University, Bangkok) for her expert views on sequence analysis.


### Conflict of interest

None declared.

### Authors' contributions

FP: performed the molecular works, interpreted data, and prepared the manuscript. EB: organised the work, collected and interpreted data, and critically revised the manuscript. TE: performed sequencing, analysed and interpreted the sequences, and revised the manuscript.

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Research article

# Filling in the gaps: estimating numbers of chlamydia tests and diagnoses by age group and sex before and during the implementation of the English National Screening Programme, 2000 to 2012

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**Citation style for this article:**
Chandra NL, Soldan K, Dangerfield C, Sile B, Duffell S, Talebi A, Choi YH, Hughes G, Woodhall SC. Filling in the gaps: estimating numbers of chlamydia tests and diagnoses by age group and sex before and during the implementation of the English National Screening Programme, 2000 to 2012. Euro Surveill. 2017;22(5):pii=30453. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.5.30453

Article submitted on 22 January 2016 / accepted on 04 October 2016 / published on 02 February 2017

**To inform mathematical modelling of the impact of chlamydia screening in England since 2000, a complete picture of chlamydia testing is needed. Monitoring and surveillance systems evolved between 2000 and 2012. Since 2012, data on publicly funded chlamydia tests and diagnoses have been collected nationally. However, gaps exist for earlier years. We collated available data on chlamydia testing and diagnosis rates among 15–44-year-olds by sex and age group for 2000–2012. Where data were unavailable, we applied data- and evidence-based assumptions to construct plausible minimum and maximum estimates and set bounds on uncertainty. There was a large range between estimates in years when datasets were less comprehensive (2000–2008); smaller ranges were seen hereafter. In 15–19-year-old women in 2000, the estimated diagnosis rate ranged between 891 and 2,489 diagnoses per 100,000 persons. Testing and diagnosis rates increased between 2000 and 2012 in women and men across all age groups using minimum or maximum estimates, with greatest increases seen among 15–24-year-olds. Our dataset can be used to parameterise and validate mathematical models and serve as a reference dataset to which trends in chlamydia-related complications can be compared. Our analysis highlights the complexities of combining monitoring and surveillance datasets.**

## Introduction

Genital infection with *Chlamydia trachomatis* ('chlamydia') is the most commonly diagnosed sexually transmitted infection (STI) in Europe [1,2]. If left untreated, genital chlamydia infection can cause serious complications in both women and men, including pelvic inflammatory disease (PID), ectopic pregnancy, tubal factor infertility and epididymitis [3]. Prevalence is generally highest in young adults [4-6]. A recent systematic review found estimates of prevalence in Europe and other high-income countries among sexually-experienced ≤ 26-year-olds ranged from 3.0% to 5.3% in women and 2.4% to 7.3% in men [7].

Chlamydia control activities vary across Europe, ranging from case management for diagnosed cases to opportunistic testing among asymptomatic individuals [8]. In England, in recognition of the public health importance of genital chlamydia infection, the National Chlamydia Screening Programme (NCSP) was introduced in 2003 and was active nationwide by 2008. The programme aims to reduce chlamydial infections and associated complications by detecting and treating asymptomatic infections through opportunistic screening. The NCSP recommends all sexually active under-25-year-olds be tested for genital chlamydia infection annually or on change of sexual partner (whichever is the most frequent) and those who test positive should be offered a re-test around 3 months after treatment [3]. Screening is accessible through a range of providers, which include general practitioners (GPs), pharmacies, contraception services, sexual health and reproductive services and pregnancy termination services [9]. In 2013, over 1.7 million chlamydia tests were carried out among 15–24-year-olds in both specialist sexual health services (genitourinary medicine clinics (GUM)) and non-specialist services with over 139,000 positive chlamydia results reported (hereafter 'diagnoses' refers to 'positive chlamydia results') [10].

Understanding changes in chlamydia tests and diagnosis will better inform us of the NCSP's effects on chlamydia infections and can facilitate programme evaluation in regards to both genital chlamydia prevalence and understanding related complications. Gaining an understanding of the NCSP's impact is also useful for other European countries considering the optimal approach to chlamydia control [11]. Annual data on testing and diagnoses are required for parameterisation of mathematical models which explore the effect of chlamydia screening on chlamydia prevalence

**Figure 1**

Schematic of available chlamydia activity data from national monitoring and surveillance systems in specialist sexual health services and non-specialist services, England, 2000–2012

<table>
<tr><td>Year</td><td></td><td>2000</td><td>2001</td><td>2002</td><td>2003</td><td>2004</td><td>2005</td><td>2006</td><td>2007</td><td>2008</td><td>2009</td><td>2010</td><td>2011</td><td>2012</td></tr>
<tr><td rowspan="2">Data captured in specialist sexual health services</td><td>Data source</td><td colspan="9">KC60 statistical return</td><td colspan="4">GUMCAD</td></tr>
<tr><td>Data available</td><td colspan="3">Number of diagnoses by sex [a]<br>Number of diagnoses in MSM<br>Number of tests not available [a]</td><td colspan="6">Number of diagnoses by sex and age group [b]<br>Number of tests and diagnoses in MSM<br>Number of tests by sex [a]</td><td colspan="4">Number of tests and diagnoses by sex, age group and sexual orientation</td></tr>
<tr><td rowspan="4">Data captured in non-specialist services</td><td>Data source</td><td colspan="3">CPRD</td><td colspan="5">NCSP statistical return & CPRD</td><td colspan="3">Aggregated laboratory returns, NCSP & CPRD</td><td>CTAD</td></tr>
<tr><td rowspan="3">Data available</td><td colspan="12">CPRD: Number of tests & diagnoses in general practice, by age and sex (15–44 year-olds)</td><td rowspan="3">Number of tests & diagnoses by sex and age (all ages)</td></tr>
<tr><td colspan="5"></td><td colspan="7">NCSP statistical returns: Number of tests & diagnoses through the NCSP by age and sex, 15–24 year-olds</td></tr>
<tr><td colspan="8"></td><td colspan="4">Aggregated laboratory returns: Number of tests and diagnoses outside NCSP by sex and age group, 15–24 year-olds</td></tr>
</table>

CPRD: Clinical Practice Research Datalink; CTAD: Chlamydia Testing Activity Dataset; GUMCAD: genitourinary medicine clinic activity dataset; MSM: men who have sex with men; NCSP: National Chlamydia Screening Programme.

GUMCAD is a collection of electronic patient-level data that captures tests and diagnoses provided in commissioned sexual health services. CTAD is a collection of electronic patient-level data that captures all publicly funded chlamydia testing activity and diagnoses in England.

[a] Includes uncomplicated and complicated chlamydia captured separately from each other in the KC60 statistical return.

[b] Includes uncomplicated and complicated chlamydia captured separately from each other in the KC60 statistical return but complicated chlamydia is not broken down by age group.

As acknowledged by authors of previous mathematical modelling studies of chlamydia screening, one of the limitations of current models is the availability of robust data on chlamydia tests and diagnoses in this context. A comprehensive overview of testing practices before and during NCSP implementation can also facilitate interpretation of trends in chlamydia-related complications. However, since 2000, national monitoring and surveillance systems that include reported chlamydia tests and diagnoses in England have evolved considerably [10,12,13] resulting in reporting gaps in the data. These gaps include non-reported data from specific years, settings or age groups; missing age and sex data; and referrals between settings. In this paper, we estimate chlamydia testing and diagnosis rates by age group among men and women aged 15 to 44 years between 2000 and 2012 using data from several datasets and a sample of GP records, combined with data-driven and evidence-based assumptions, to fill the gaps in these data. Our estimates of chlamydia testing and diagnosis rates are needed to provide robust data for mathematical models that can be used to evaluate chlamydia control activities.

# Methods

## Data sources

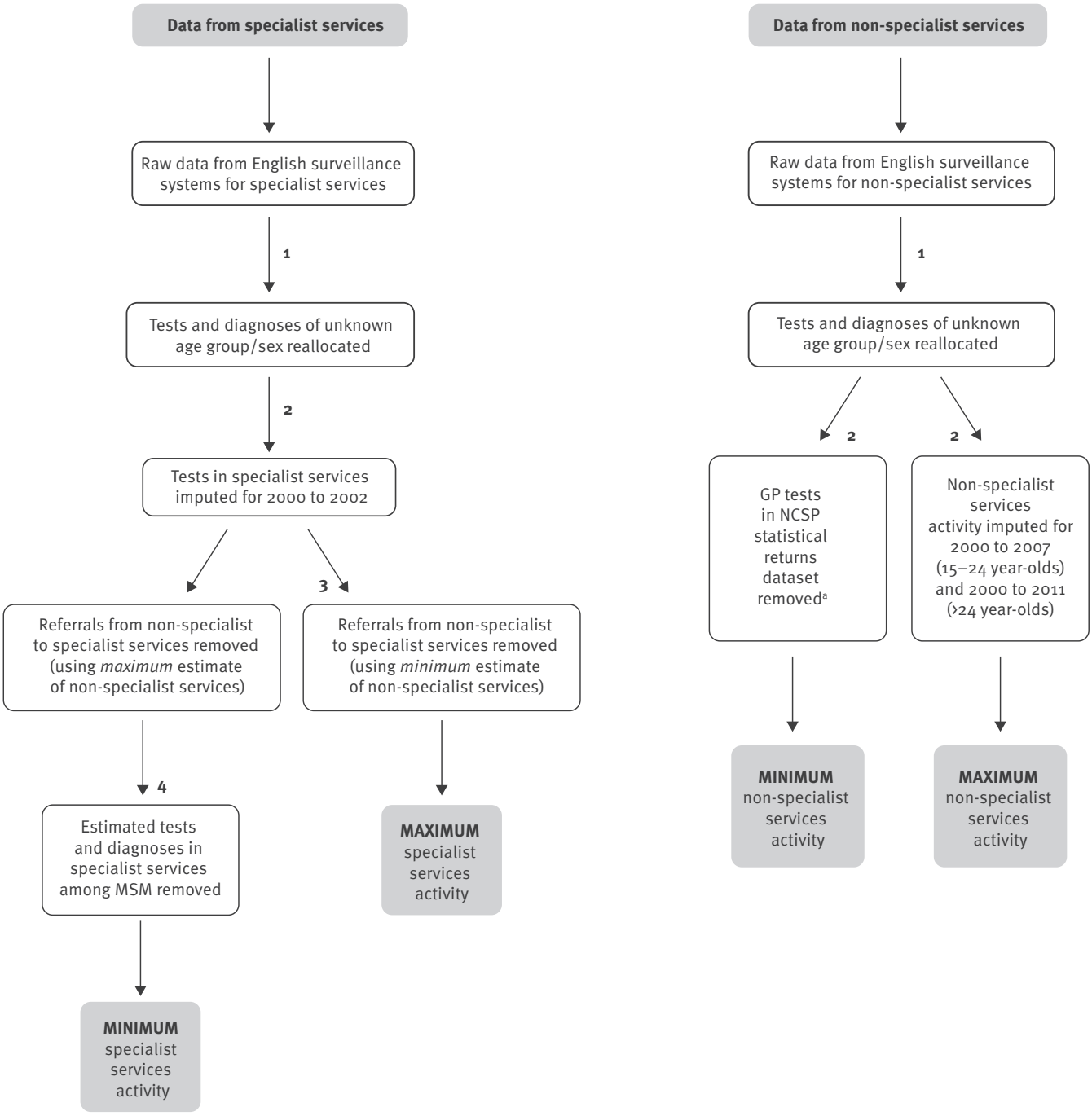
Data on chlamydia tests and diagnoses were compiled from several data sources. The data available varied according to the years and test settings covered and the extent to which data were provided by age group (Figure 1).

## Specialist sexual health services

Numbers of chlamydia tests and diagnoses carried out in specialist sexual health services were derived from the KC60 statistical return (2000–2008) and genitourinary medicine clinic activity dataset (GUMCAD, 2009–2012). Details of these datasets are reported elsewhere [12]. Briefly, the KC60 was a mandatory statistical return which provided an aggregated dataset of diagnoses and services delivered in specialist sexual health services in England up to 2008. Between 2000 and 2002 the number of tests were not recorded; diagnoses were broken down by sex but not age. Tests were included from 2003 and broken down by sex but not age; diagnoses were available by sex and age for

**Figure 2**

Flowchart summarising combinations and adjustments to the data from specialist sexual health services and non-specialist sexual health services to construct plausible minimum and maximum estimates of chlamydia tests and diagnosis rates by sex and age group, England, 2000–2012




GP: general practice; MSM: men who have sex with men; NCSP: National Chlamydia Screening Programme.

Adjustment number 1: dealing with data of unknown age group or sex; Adjustment number 2: imputing non-reported data from a specific year, setting or age group; Adjustment number 3: allowing for referrals from non-specialist to specialist settings; Adjustment number 4: removal of MSM from the minimum-activity estimate for specialist settings.

[a] It is unknown whether chlamydia tests carried out in GP settings, but as part of the NCSP, would also be captured within GP records. Therefore, where there was potential duplication (2003–2007), GP tests were removed from the NCSP statistical returns dataset.

uncomplicated chlamydial infections but not available by age for complicated chlamydial infections. GUMCAD, which was introduced in 2009, is a mandatory disaggregated data return of STI diagnoses and services provided submitted by all specialist services across England [12]. The number of tests and diagnoses are available by sex, age and sexual orientation.

## Non-specialist sexual health services

Chlamydia tests and diagnoses made outside specialist sexual health services were derived from three nationally collated datasets:

- NCSP statistical returns (2003–2011): a disaggregated return from testing venues of all chlamydia tests and diagnoses among 15–24-year-olds tested as part of the NCSP between 2003 and September 2012.

- Aggregated laboratory return (2008–2011): this return captured data from all laboratories that collected tests and diagnoses among 15–24-year-olds reported from outside of specialist sexual health services and not as part of the NCSP between April 2008 and September 2012 (e.g. in hospitals or in GP settings not carried out as part of the NCSP).

- Chlamydia Testing Activity Dataset (CTAD, 2012): CTAD is a disaggregated data return from laboratories that replaced the NCSP statistical return and aggregated laboratory return in 2012. CTAD captures all publicly funded chlamydia tests and diagnoses in England for all ages [10,13].

Before the introduction of CTAD in 2012, national monitoring and surveillance systems in England did not cover chlamydia testing carried out among those aged 25 years and over attending non-specialist clinics [12,13]. We therefore supplemented the datasets described above using data from the clinical practice research datalink (CPRD). CPRD comprises anonymised patient-level medical records of registered patients in a sample (ca 10%) of GPs across the United Kingdom (UK) [14-16]. Attendances for chlamydia tests and diagnoses among men and women aged 15–44 years between 2000 and 2011 were identified using a predefined selection of 'Read Codes' (diagnostic codes used in primary care, data not shown). Duplicate codes within a 42-day period were considered part of the same episode and subsequently excluded.

With the exception of data from CPRD, estimates of testing coverage (number of tests as a percentage of the population) and diagnosis rates (number of diagnoses per 100,000 population) were calculated using population denominators provided from the Office of National Statistics [17]. Testing and diagnosis rates reported in CPRD were calculated by dividing the number of tests and diagnoses by the person-years contributed by the registered practice population in each year by sex and age group [14].

## Data and evidence base to address the known limitations of national monitoring and surveillance systems

After combining the datasets, we made a series of adjustments to resolve gaps in the data and calculate minimum and maximum plausible estimates of chlamydia testing coverage and diagnosis rates for each age group by sex and year in both specialist and non-specialist services (Figure 2). We define adjustments to the data as modifications (as described below) rather than statistical adjustments. The datasets were adjusted to account for missing age and sex variables, differences in case definitions (complicated and uncomplicated chlamydial infections), referrals between non-specialist and specialist services, and missing data for certain years. Some adjustments to the data could be undertaken using more than one possible method. To justify the methods used, sensitivity analyses, statistical tests and comparisons to other research were used (Table 1).

## Unknown age group or sex

Due to missing fields or aggregated reporting, tests and diagnoses could be reported without known sex or age group, therefore in these instances tests and diagnoses were reallocated according to the age and sex distributions seen in each year (see adjustment number 1 in Figure 2 and Table 1).

Between 2000 and 2008, diagnoses coming from specialist services were coded as either 'uncomplicated' or 'complicated' chlamydia (i.e. complicated when diagnosed with chlamydial PID and epididymitis). Complicated chlamydia diagnoses were not reported by age group. Based on a two-sample Kolmogorov-Smirnov test, the age distributions for 'complicated' and 'uncomplicated' chlamydia diagnoses in specialist services between 2009 and 2011 were not significantly different (borderline, $p = 0.053$). We therefore assumed the distributions were not significantly different between 2000 and 2008 and the 'complicated' diagnoses were reallocated into age groups according to the age distribution of 'uncomplicated' diagnoses.

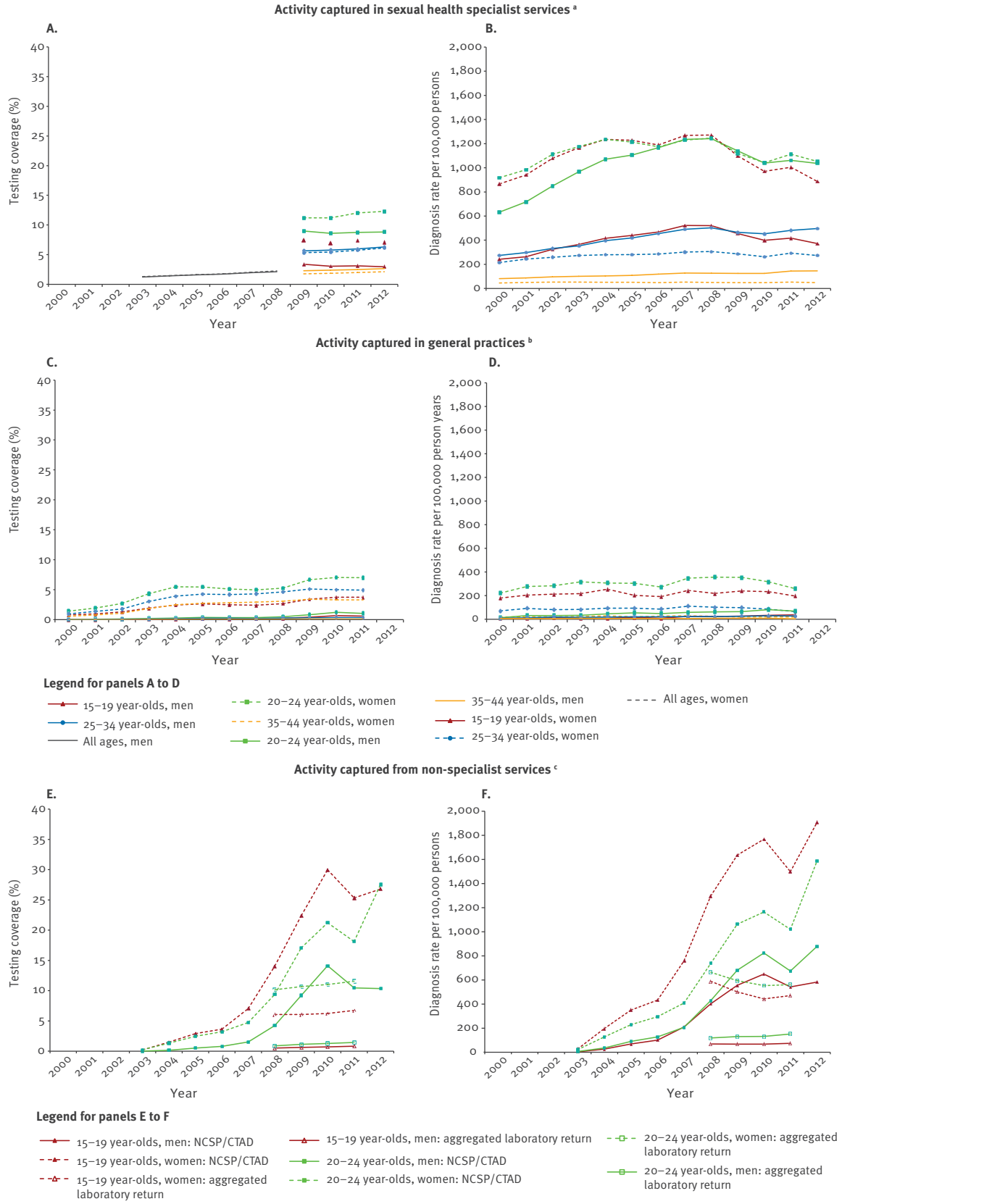
Between 2003 and 2008, chlamydia tests in specialist services were reported by sex but not by age group. We therefore reallocated tests reported during this period into age groups according to the age group distribution of tests in 2009. This is based on analysis of variance tests (ANOVA) showing a non-significant difference between the age distribution of diagnoses between 2003 and 2012 ($p = 1.0$) and a non-significant difference between the age distribution of tests between 2009 and 2012 (the years where tests by age were reported, $p = 0.9$).

## Non-reported data: age group

During the analysis period, there are two major gaps in reporting where no data were collected through national monitoring and surveillance systems: (i) Before 2003, the number of chlamydia tests in specialist services

## Figure 3

Reported rates of chlamydia tests and diagnoses captured in specialist sexual health services and non-specialist services by sex and age group, Panels A), C) and E) show tests; Panels B), D) and F) show diagnosis rates, England, 2000–2012




CPRD: Clinical Practice Research Datalink; CTAT: Chlamydia Testing Activity Dataset; GUMCAD: Genitourinary Medicine Clinic Activity Dataset; NCSP: National Chlamydia Screening Programme.

[a] All ages for tests done in specialist services include 15- to 90-year-olds; testing activity was not broken down by age pre-2009. Diagnoses captured in specialist services incorporate both uncomplicated and complicated chlamydia diagnoses. Testing data in specialist services are available from 2003 to 2012 and diagnosis data are available from 2000 to 2012.Data captured in specialist services are from the English monitoring and surveillance systems: KC60 statistical return (2000 to 2008) and GUMCAD (2009 to 2012).

[b] Activity rates within general practices are by person-years; data captured from 2000 to 2011 are for 15 to 44-year-olds. Data for general practices are captured in CPRD (2000 to 2011).

[c] Testing and diagnosis data in non-specialist services capture data for 15 to 24-year-olds only. Data captured in non-specialist services are from English monitoring and surveillance systems: NCSP statistical return (2003 to 2011), aggregated laboratory return (2008 to 2011) and CTAD (2012). CTAD captures data for all ages, only data for 15 to 24-year-olds are displayed here.

**Table 1**

Rationale for methods used for adjustments to the data for estimating numbers of chlamydia tests and diagnoses by age group and sex, before and during the implementation of the National Screening Programme, England, 2000–2012.

| Adjustment and adjustment number[a] | Assumption | Data and evidence base for assumption |
|---|---|---|
| Reallocation of **complicated** chlamydia diagnoses into age groups. Adjustment number: 1 | Assumes that the age distribution of diagnoses for complicated chlamydia in 2000 to 2008 was equivalent to that seen for uncomplicated chlamydia during the same period. | 1) A Kolmogorov-Smirnov test was used to statistically compare the uncomplicated and complicated chlamydia diagnosis distributions. This test showed no significant difference between distributions. 2) Alternatively, we could have reallocated complicated diagnoses captured between 2000 and 2008 according to the distribution of complicated diagnoses found in 2009. However, the results of a sensitivity analysis showed limited difference between methods (maximum percentage difference of 0.3% (range of 0.04–0.3%)). |
| Reallocation of **tests** between 2003 and 2008 according to the age group distribution in 2009. Adjustment number: 1 | Assumes that the age distribution of tests between 2003 and 2008 was equivalent to that seen in 2009. | The rationale for this is based on two other observations: 1) The age distribution for chlamydia diagnoses coming from specialist services between 2003 and 2008 were comparable to the age distribution of diagnoses in 2009 (ANOVA test non-significant). 2) There was no variation in the age distribution for chlamydia tests coming from specialist services between 2009 and 2012 (ANOVA test non-significant). |
| Imputing data for 15 to 24-year-olds before 2008 and for›24–year-olds before 2012 in **non-specialist** services. Adjustment number: 2 | Assumes that all testing in non-specialist services for 15–24-year-olds before 2008, and in›24-year-olds before 2012 followed a similar trend to that found in GP services. | We considered it a reasonable assumption that any changes seen in GP settings would also be reflected in other non-specialist services. Alternatively, we could have based this on the trend seen in specialist services. However, results from an audit of waiting times in specialist services show large increases in access to specialist services over this period following the first National Sexual Health Strategy [18]. Increases in testing outside of specialist services are therefore unlikely to have been of the same magnitude. |
| Imputing the number of chlamydia **tests** in **specialist** services between 2000 and 2002. Adjustment number: 2 | Assumes a consistent trend in positivity over time from 2000 to 2008. | This adjustment was based patterns seen within later years of the data. The trend in positivity on which this adjustment was made was calculated using 2003–2008 data, rather than 2003–2012, being the period before full implementation of the NCSP and the GUMCAD surveillance system, which may have led to some changes in the available data. |
| Allowing for **referrals** from non-specialist to specialist services. Adjustment number: 3 | Assumes a constant rate of referrals between non-specialist to specialist services between 2000 and 2012. | Our assumption is consistent with a previous study [21], which reported a steady referral rate between 2000 and 2004 from GP settings into specialist services. |

ANOVA: analysis of variance; GP: general practice; GUMCAD: Genitourinary Medicine Clinic Activity Dataset; NCSP: National Chlamydia Screening Programme.

[a]Adjustment number refers to the numbers found in the flowchart in Figure 2.

were not collected; and (ii) in non-specialist services, data on chlamydia tests and diagnoses were incomplete before 2012, this included non-reported data for 15–24-year-olds before 2008 and for›24-year-olds before 2012. To produce plausible estimates of total activity during these periods we imputed these data (see adjustment number 2).

Firstly, in order to impute the number of chlamydia tests in specialist services between 2000 and 2002, we used logistic regression to estimate the linear trend in positivity (percentage of chlamydia tests resulting in a positive diagnosis) between 2003 and 2008. Using the trend in positivity observed between 2003 and 2008, we predicted the positivity for 2000 to 2002. The model-predicted positivity trends were applied to the estimated diagnoses in order to estimate the numbers of tests in each year and age group from 2000 to 2002.

Secondly, we constructed minimum and maximum estimates of chlamydia testing coverage and diagnosis rates carried out in non-specialist services for 2000 to 2011 to allow for the uncertainty arising from non-reported data. Minimum estimates of chlamydia testing coverage and diagnosis rates were based on data available in the datasets (NCSP statistical returns and aggregated laboratory returns), combined with test and diagnosis rates derived from GP settings (CPRD) in those years where data were incomplete. To estimate maximum activity in non-specialist services, we used Poisson regression to estimate trends in test and diagnosis rates in the period where data from non-specialist services were incomplete or not reported. We then applied these model-estimated incidence rate ratios to the most recent 'complete' year of non-specialist services data (2008 for 15–24-year-olds; 2012 for›24-year-olds).

## Referrals from non-specialist to specialist services

Individuals cannot be followed between non-specialist and specialist services in the datasets as different identifiers are used. Since 2012, a diagnostic code to indicate referrals from non-specialist services with a

chlamydia diagnosis into specialist services was introduced (C4X code) [18,19]. We calculated the proportion of referrals in 2012, which ranged from 3.8% to 15.6% by age group. In both the minimum and maximum estimates of activity in specialist services, testing coverage and diagnosis rates were adjusted to allow for potential duplication between services, based on the proportions of referrals in 2012, assuming the rate of referrals was constant across the period (see adjustment number 3). While it is feasible that this has changed, this was considered a reasonable assumption as Hughes et al. reported a steady referral rate of 10% in 2000 to 2004 from GPs into specialist services [20], which is similar to the overall referral rate calculated for 2012 (8.4%).

### Men who have sex with men

This dataset was compiled with an aim to mathematically model heterosexual transmission of chlamydia. MSM were therefore removed from the minimum-activity estimate for specialist services (see adjustment number 4). Sexual orientation is not available for tests and diagnoses outside of specialist services, so this could not be adjusted for.

## Results

Figure 3 shows the chlamydia testing and diagnosis rates by services according to the years and age groups before adjustments were made to the data.

Table 2 shows the compiled data sources for all genital chlamydia testing and diagnosis activity by age group and sex.

Between 2000 and 2008, there was a large range between minimum and maximum estimate scenarios for both testing coverage and diagnosis rates. For example, in 15–19-year-old women in 2000, diagnosis rates ranged from 891 to 2,489 diagnoses per 100,000 persons. In both scenarios and across all age groups (15–44-year-olds), estimated testing coverage and diagnosis rates were higher in women than men.

In women and men of all age groups (15–44-year-olds), there was an overall increase in chlamydia testing coverage and diagnosis rates from 2000 to 2012 in all settings. The greatest increases in both testing coverage and diagnosis rates were seen among 15 to 24-year-olds, with the greatest increase in this age group found between 2008 and 2010. From 2010 there was a small decline in testing and diagnosis rates among 15–24-year-olds. Whereas the minimum estimate scenario showed a large increase in estimated diagnosis rates in women from 2000, a more gradual increase was seen for the maximum estimate scenario. In both minimum and maximum estimate scenarios, estimated diagnosis rates were relatively stable from 2008 to 2012 in women and men.

## Discussion

We used data captured by a range of monitoring and surveillance systems to construct a dataset representing all genital chlamydia testing and diagnosis activity taking place in England between 2000 and 2012. Gaps in the available data mean there is considerable uncertainty around the total amount of testing and diagnoses in the years before 2008. We therefore constructed minimum and maximum estimates to acknowledge this but set bounds on the uncertainty within the data.

The changes seen in chlamydia testing and diagnosis rates are in line with the evolution of the NCSP and chlamydia testing in England. An overall increase in testing and diagnosis rates were observed among 15–44-year-olds, which is likely due to increased awareness and better practice of chlamydia testing in England. The greatest increase in rates were observed in 15–24-year-olds, relating to an increase in opportunistic testing targeted in under-25-year-olds, as part of the NCSP from 2003. A sharp increase was seen from 2008 due to the nationwide implementation of the NCSP in 2008, accompanied by national targets for testing coverage. The decline in testing rates from 2010 may be explained, in part, by the changes in targets for testing during this period [21].

The constructed dataset resulting from our work has several applications. From these findings we have a better understanding of the potential effects of the NCSP on testing coverage and diagnoses. However, this does not provide the complete picture, as further insight is needed to understand how prevalence and/or incidence have changed in the context of the programme. Mathematical modelling offers a means to do this and our constructed data can be used to parameterise such models to better quantify the public health impact of the NCSP. Our data can also be used to parameterise and validate mathematical models designed to explore optimum approaches to chlamydia control (e.g. by varying rates of partner notification or changing the population tested). The findings of such modelling would be of benefit beyond England as the principles of chlamydia epidemiology and likely impact of different chlamydia control measures would likely hold across many different countries. Findings from such analyses could therefore inform chlamydia control activities in Europe and elsewhere.

In addition, these data can serve as a reference for interpreting trends in chlamydia-related complications. For example, trend in rates in PID, a complication associated with STIs including chlamydia, can be compared with chlamydia rates and determine if any changes reflected in one may be reflected in the other [22]. This is important for evaluation of the NCSP as an aim of the programme is to reduce associated complications through opportunistic screening. Again, findings from such studies would have relevance beyond England, as a better understanding the impact of chlamydia control

## Table 2

Minimum and maximum estimates for chlamydia testing coverage and diagnosis rates in 15 to 44-year-old women and men across all service types.

| | | Chlamydia testing coverage in women (per 100 persons) | | | | | | | | Chlamydia testing coverage in men (per 100 persons) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Minimum estimates | | | | Maximum estimates | | | | Minimum estimates | | | | Maximum estimates | | | |
| | | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds |
| Year | 2000 | 3.1 | 5.5 | 2.7 | 1.1 | 9.7 | 11.1 | 5.4 | 2.4 | 1.1 | 2.9 | 2.0 | 0.8 | 2.0 | 3.9 | 2.5 | 1.0 |
| | 2001 | 3.8 | 6.6 | 3.6 | 1.5 | 11.1 | 12.8 | 6.2 | 2.7 | 1.3 | 3.5 | 2.2 | 0.8 | 2.4 | 4.7 | 2.8 | 1.2 |
| | 2002 | 4.8 | 8.5 | 4.3 | 1.9 | 13.0 | 14.9 | 7.0 | 3.1 | 1.6 | 4.4 | 2.6 | 1.0 | 3.1 | 5.8 | 3.3 | 1.4 |
| | 2003 | 6.3 | 11.2 | 6.0 | 2.8 | 14.9 | 17.1 | 7.9 | 3.5 | 2.0 | 5.4 | 3.0 | 1.1 | 3.8 | 7.0 | 3.6 | 1.5 |
| | 2004 | 8.5 | 14.1 | 7.3 | 3.6 | 16.8 | 19.3 | 8.9 | 3.9 | 2.4 | 6.2 | 3.5 | 1.3 | 4.7 | 8.1 | 4.3 | 1.8 |
| | 2005 | 10.3 | 15.6 | 8.0 | 4.0 | 19.0 | 21.5 | 9.9 | 4.4 | 3.1 | 7.1 | 4.0 | 1.5 | 5.6 | 9.2 | 4.8 | 2.0 |
| | 2006 | 11.1 | 16.3 | 8.2 | 4.1 | 21.2 | 23.8 | 10.9 | 4.9 | 3.5 | 7.8 | 4.3 | 1.6 | 6.8 | 10.4 | 5.4 | 2.2 |
| | 2007 | 15.1 | 18.7 | 9.0 | 4.5 | 24.2 | 27.2 | 12.4 | 5.5 | 5.5 | 9.3 | 4.9 | 1.8 | 8.4 | 12.1 | 6.2 | 2.5 |
| | 2008 | 27.3 | 30.4 | 9.9 | 4.8 | 27.3 | 30.4 | 13.8 | 6.2 | 10.2 | 13.4 | 5.3 | 2.0 | 10.4 | 13.9 | 6.8 | 2.8 |
| | 2009 | 35.7 | 38.8 | 10.4 | 5.2 | 35.7 | 38.8 | 14.8 | 6.8 | 16.1 | 18.8 | 5.3 | 2.0 | 16.3 | 19.3 | 7.1 | 2.9 |
| | 2010 | 43.0 | 43.3 | 10.4 | 5.2 | 43.0 | 43.3 | 16.0 | 7.5 | 23.9 | 23.3 | 5.4 | 2.1 | 24.0 | 23.9 | 7.5 | 3.1 |
| | 2011 | 39.3 | 41.6 | 10.6 | 5.3 | 39.3 | 41.6 | 17.5 | 8.4 | 19.0 | 19.8 | 5.4 | 2.1 | 19.2 | 20.6 | 8.1 | 3.4 |
| | 2012 | 33.9 | 39.9 | 19.2 | 9.3 | 33.9 | 39.9 | 19.2 | 9.3 | 14.2 | 18.2 | 7.7 | 3.0 | 14.5 | 19.2 | 8.8 | 3.7 |
| | | Chlamydia diagnosis rates in women (per 100,000 persons) | | | | | | | | Chlamydia diagnosis rates in men (per 100,000 persons) | | | | | | | |
| | | Minimum estimates | | | | Maximum estimates | | | | Minimum estimates | | | | Maximum estimates | | | |
| | | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds | 15–19-year-olds | 20–24-year-olds | 25–34-year-olds | 35–44-year-olds |
| Year | 2000 | 891.1 | 1,043.1 | 256.7 | 56.5 | 2,488.6 | 1,909.5 | 502.6 | 113.3 | 235.7 | 621.8 | 268.1 | 78.8 | 387.1 | 815.7 | 343.4 | 113.5 |
| | 2001 | 987.5 | 1,162.3 | 305.5 | 62.9 | 2,589.5 | 2,012.8 | 530.8 | 117.2 | 255.7 | 717.7 | 291.7 | 81.8 | 431.7 | 925.5 | 372.9 | 120.8 |
| | 2002 | 1,130.7 | 1,293.3 | 311.3 | 67.0 | 2,756.4 | 2,182.7 | 548.9 | 120.2 | 318.6 | 849.1 | 330.6 | 87.8 | 519.3 | 1,088.3 | 412.1 | 129.4 |
| | 2003 | 1,244.1 | 1,402.0 | 327.8 | 68.3 | 2,871.2 | 2,288.0 | 564.6 | 120.0 | 356.3 | 969.4 | 345.8 | 94.0 | 589.8 | 1,241.3 | 440.2 | 134.6 |
| | 2004 | 1,508.3 | 1,538.9 | 344.7 | 73.4 | 2,952.0 | 2,388.4 | 572.3 | 118.4 | 424.5 | 1,108.8 | 390.5 | 94.8 | 674.9 | 1,383.8 | 490.9 | 138.5 |
| | 2005 | 1,576.5 | 1,588.3 | 344.6 | 64.6 | 2,968.4 | 2,408.1 | 574.8 | 118.9 | 489.4 | 1,189.2 | 408.4 | 97.2 | 736.8 | 1,460.3 | 521.0 | 143.7 |
| | 2006 | 1,594.2 | 1,568.0 | 340.3 | 68.7 | 2,954.7 | 2,417.4 | 581.6 | 115.0 | 535.0 | 1,267.3 | 439.2 | 103.8 | 811.2 | 1,572.9 | 565.8 | 155.2 |
| | 2007 | 2,022.5 | 1,782.3 | 381.3 | 74.7 | 3,034.0 | 2,510.8 | 597.1 | 119.4 | 704.0 | 1,415.6 | 474.6 | 110.7 | 912.5 | 1,696.3 | 611.7 | 164.2 |
| | 2008 | 2,979.6 | 2,516.9 | 379.3 | 68.4 | 2,979.6 | 2,516.9 | 604.0 | 117.3 | 946.9 | 1,716.1 | 477.6 | 105.4 | 957.6 | 1,751.5 | 632.6 | 163.7 |
| | 2009 | 3,033.9 | 2,621.4 | 355.2 | 59.8 | 3,033.9 | 2,621.4 | 587.2 | 116.4 | 1,020.2 | 1,854.3 | 435.3 | 99.8 | 1,032.6 | 1,895.4 | 605.8 | 163.5 |
| | 2010 | 2,972.8 | 2,598.7 | 318.9 | 61.5 | 2,972.8 | 2,598.7 | 566.2 | 115.8 | 1,049.4 | 1,879.1 | 416.3 | 94.5 | 1,063.8 | 1,928.0 | 606.0 | 163.2 |
| | 2011 | 2,786.6 | 2,547.1 | 327.2 | 64.9 | 2,786.6 | 2,547.1 | 599.3 | 120.0 | 970.9 | 1,754.0 | 414.8 | 91.4 | 989.9 | 1,829.5 | 647.0 | 184.1 |
| | 2012 | 2,791.8 | 2,639.5 | 587.6 | 117.3 | 2,791.8 | 2,639.5 | 587.6 | 117.3 | 935.5 | 1,830.2 | 589.7 | 130.0 | 953.8 | 1,913.7 | 678.7 | 186.8 |

on complications is needed to inform decisions about how best to approach chlamydia control [11].

The main strength of our analysis is the use of data from well-documented and established datasets, in which the changes in coding, testing practices and gaps in the data are understood. There are, however, some limitations. While every effort has been made to use data-driven and evidence-based assumptions to adjust for missing data, it is possible that our estimates have resulted in some over- or under- estimation of activity. We used data on referral patterns in 2012 to de-duplicate testing episodes between settings. However de-duplication of testing or diagnosis episodes is likely to be incomplete. For example, if an individual visited two different specialist services for the same testing episode, it would not be possible to remove the duplicate record. For this analysis, only tests undertaken by publicly-funded services have been counted, as private tests are excluded as part of the data collection specification [13]. When dealing with data where the age and sex were unknown, we used statistical tests to guide our decision about the most appropriate distribution to apply to the data. In the case of complicated chlamydia diagnoses, our finding was of borderline significance meaning that we may have incorrectly allocated by age group. However, as complicated diagnoses made up a minority of diagnoses from specialist sexual health services over this period (<3.5%) this is unlikely to have made a substantial difference to the resulting dataset. Sensitivity analysis showed that applying an alternative assumption (i.e. reallocate according to the age distribution seen in 2009) made negligible difference. It is feasible that reallocating tests of unknown age in 2003 to 2008 according to the age-group distribution of tests in 2009 may have introduced error, as the NCSP was being rolled out in these earlier years.

During the analysis period, more sensitive and specific chlamydia tests have become available [23]. There is potential for both false negative and false positive results to have occurred over this period due to imperfect sensitivity and specificity of enzyme immunoassay (EIA) tests in particular, which were phased out in England during the mid-2000s [23]. We did not adjust our estimated diagnosis rates for test performance, as the test platforms used were not routinely collected and the exact performance characteristics are difficult to apply given the absence of an agreed gold standard [24].

Given the nature of this work and the absence of data, there are limited sources in the literature to validate our estimates. However, findings from the second National Survey of Sexual Attitudes and Lifestyles (Natsal-2, a stratified probability survey of British general population carried out in 1999–2000) are consistent with the estimated diagnosis rates calculated in this work. For example among 20–24-year-old Natsal-2 participants who had ever had sex, 0.7% (95% confidence interval 0.2–2.0%) of men and 1.7% (1.0–2.9%) of women reported having been diagnosed with chlamydia in the last year [25]. In our constructed dataset, assuming that each diagnosis represents an individual, the minimum and maximum estimates of percentage tested in 2000 was 0.6% to 0.8% in 20–24-year-old men and 1.0% to 1.9% in women, thus falling within the 95% confidence limits of the survey-based estimates. Currently, we do not have other external validation methods.

While there is uncertainty in the absolute numbers of chlamydia tests and diagnoses estimated in the earlier years of our analysis period, it is highly likely that testing and diagnosis rates did increase from at least the early 2000s onwards. This is especially the case among under-25-year-olds as the target age group for the NCSP, which was implemented on a phased basis in 2003 and achieved national implementation by 2008 [9,26]. Data from the second and third Natsal studies in 1999–2000 (Natsal-2) and 2009–2011 (Natsal-3)) indicate that diagnoses have increased substantially over the decade, with the percentage of 16–24-year-olds who reported a chlamydia diagnosis in the last 5 years increasing from 1.5% (1.2–1.8) to 4.1% (3.6–4.7) in women and from 0.8% (0.5–1.1) to 4.0% (3.4–4.8) in men [4]. It is likely therefore that our maximum scenario estimates of diagnoses in earlier years in women are an overestimate. However, we retained this liberal estimate of diagnoses in the maximum scenario as we could not narrow these plausible ranges further on the basis of the available data.

The problem of missing data from chlamydia surveillance systems is not one limited to England. Surveillance systems across Europe are known to vary in their completeness with respect to diagnoses, and few countries routinely collect and report data on testing, which is invaluable in interpreting trends in diagnoses of chlamydia, given that it is a largely asymptomatic infection. So, could our approach be applied to other settings? Our analysis has highlighted the multiple complexities in undertaking such an exercise, even in the context of England, where surveillance systems are more complete than many others in Europe and have included testing denominators for several years [8]. However, it is possible that multiple data sources from other countries may be combined in a similar fashion to ascertain minimum and maximum estimates, through application of reasonable assumptions about the completeness of the data or relationships between them. Such an undertaking would need to be carried out on a case by case basis, involving in-country experts with in-depth knowledge of data collection systems as well as an understanding of healthcare systems and changes in policy and practice over time.

## Conclusions

Our analysis provides plausible comprehensive estimates of chlamydia testing and diagnosis activity in England from 2000. Since 2012, developments in monitoring and surveillance systems for chlamydia and other STI in England, embodied by CTAD and

GUMCADv2, have allowed a comprehensive record of chlamydia testing and diagnosis activity from a single data source with far less uncertainty, enabling more robust assessment and evaluation of the English NCSP in future years. It is possible that similar methods to ours could be used for data captured in surveillance systems in applicable countries across Europe, however, our analysis highlights the potential complexities faced when estimating testing and diagnosis activity from multiple and changing data sources. When examining trends over time using monitoring and surveillance data or compiling data from different sources, we recommend that known limitations be carefully considered and addressed where possible.

## Conflict of interest

None declared.

## Authors' contributions

SW and KS had the original idea for the study. NC and SW compiled the data. NC, SW, KS, CD, YC, and GH advised on the methods for compiling the data. SD, BS, and AT provided data from the original surveillance systems and advised on their use. NC wrote the first draft of the paper. All authors contributed to the writing of the manuscript and approved the final version.

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