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Response to a wild poliovirus type 2 (WPV2)-shedding event following accidental exposure to WPV2, the Netherlands, April 2017

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On 3 April 2017, a wild poliovirus type 2 (WPV2) spill occurred in a Dutch vaccine manufacturing plant. Two fully vaccinated operators with risk of exposure were advised on stringent personal hygiene and were monitored for virus shedding. Poliovirus (WPV2-MEF1) was detected in the stool of one, 4 days after exposure, later also in sewage samples. The operator was isolated at home and followed up until shedding stopped 29 days after exposure. No further transmission was detected.

The Dutch National Polio Laboratory of the National Institute of Public Health and the Environment (RIVM) was informed about a partly aerosolised high titre spill of monovalent wild poliovirus type 2 (WPV2-MEF1) in a vaccine manufacturing plant in the centre of the Netherlands on 3 April 2017. Following the World Health Organization (WHO) recommendations [1], all staff working with infectious polioviruses have to be vaccinated, however, vaccines (inactivated polio vaccine (IPV) and oral polio vaccine (OPV)) protect against disease, not against infection. Therefore, all staff who might have been exposed needed to be followed up to check for infection and excretion of the virus. Two fully vaccinated operators with possible exposure related to the event were identified and monitored. Immediately after the detection of poliovirus in the stool of the one of them on 7 April, the Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) formed an outbreak management team to closely monitor the situation, and to enforce and facilitate stringent hygiene measures and voluntary home isolation. Here we describe the response to the event including considerations and readjustments of follow-up measures, according to newly available information.

Containment and monitoring

Exposed operators

Following the possible exposure, the two fully vaccinated operators were monitored according to the protocol of the facility. Advice on stringent personal hygiene was given on the day of the incident, as well as instructions to avoid contact with unvaccinated persons. Throat swabs and stool specimens were collected on day 3/4 and day 7/8 after exposure. On Friday, 7 April, (day 4 after exposure), throat and stool samples were collected from both operators and these were analysed by RT-PCR. The faecal sample of one of the exposed operators was positive for poliovirus by RT-PCR. On Sunday, 9 April, virus cultures on L20B and rhabdomyosarcoma (RD) cells were also positive and the samples were processed for full poliovirus VP1 sequencing. On 10 April, sequencing of this stool sample showed 100% identity of full VP1 to WPV2 (MEF-1 IPV strain) (data not shown). On the same day, this confirmation of a WPV2 infection in an operator in the Netherlands was reported to the WHO, according to the International Health Regulations [2]. The European Commission and relevant authorities in the European Union (EU) Member States were informed through the EU Early Warning and Response System (EWRS).

Throat swabs of the infected operator collected on day 4 and 8, as well as all samples of the second operator, remained negative in RT-PCR on clinical material and in virus isolation.

Diagnostic procedures and laboratory containment

Samples received on 7 April were processed in the National Polio Laboratory at the RIVM under poliovirus-containment-approved biosafety level (BSL)-2 conditions. From 11 April, all samples of the infected operator

were processed under BSL-3 containment, including the use of filtering face piece (FFP)3 masks and excluding the presence of other staff. All other samples (from contacts and sewage) were initially processed under BSL-2 conditions. As soon as cultures started showing cytopathogenic effects (CPE) indicative of virus propagation, the closed culture tubes were transported to the BSL-3 laboratory.

All samples in this monitoring programme were analysed by RT-PCR for generic enterovirus and poliovirus detection and specific WPV2 detection and all samples were processed for virus isolation according to the WHO protocol [3]. The cultures of the infected operator were disposed of (following BSL-3 waste management guidelines) as soon as CPE appeared and the WPV2 PCR on the stool suspension was positive, except for six samples. For these six samples, the L20B cultures were opened and viral RNA was extracted for sequencing. The sewage samples were processed as described previously [4] under BSL-2 containment. All laboratory staff who processed WPV2-positive materials under BSL-2 containment were followed up by day 3/4 and day 7/8 stool sample analysis. All remained negative for WPV2 excretion.

Follow-up of the infected operator

The infected operator was followed up by daily stool sampling. This was continued until the stool tested negative for at least 3 consecutive days, which is in line with the WHO 'Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use' (GAP III) [1].

Starting with 7 April, the infected operator was signed off from work and in voluntary home isolation under daily supervision of the local public health service. The infected operator resides in an area of the Netherlands with high vaccination coverage, which does not belong to the so-called Bible belt, an area where some inhabitants object to vaccination on religious grounds and that has a lower coverage [5,6]. Until 13 April, the infected operator used the sole toilet in their apartment together with two household members. The infected operator was instructed by the local public health service to follow strict hygiene measures such as flushing with toilet lid closed, disinfection with chlorine after every defecation and strict hand hygiene (i.e. using medical gloves while using the toilet, sampling and disposing of faeces, and washing hands afterwards). Starting with 14 April, all stools from the infected operator were collected in a disposable system as the one recommended for Ebola virus disease patients [7]: a toilet chair, a plastic bag with absorbent material, in a plastic waste container. These disposable materials were disposed of in a high-level containment box before transportation to and immediate destruction at the designated waste incineration plant. The infected operator's stools tested positive until 29 April and once again on 1 May (Figure). They were in home isolation for 32 days.

Follow-up of household contacts

An investigation of the contacts was undertaken on 8 April, revealing four persons who had been in the house of the infected operator in the previous days (two visitors and two household contacts). All were fully vaccinated with IPV according to the national immunisation schedule. The two visitors visited the infected operator on 7 and 8 April (one of them visited on both days); both were monitored by stool and throat samples collected on day 4 and day 6/8 after contact. All samples of these two contacts were negative for poliovirus and monitoring was stopped (Figure).

The household members were followed up by throat swabs and stool sampling starting with day 3/4 following the first possible exposure (Figure). Monitoring of the two household members was continued until 10 days after the end of virus shedding by the infected operator. As at 12 May, all samples were negative by RT-PCR and monitoring was stopped.

Advice on personal hygiene was provided to all contacts as well as instructions to avoid contact with unvaccinated persons, and to refrain from visiting regions with low vaccination coverage and from specific activities such as swimming. The two household members were explicitly requested not to defecate outside their home. Apart from the two visitors and the two household members, no visitors were allowed to enter the home of the infected operator while they were shedding virus.

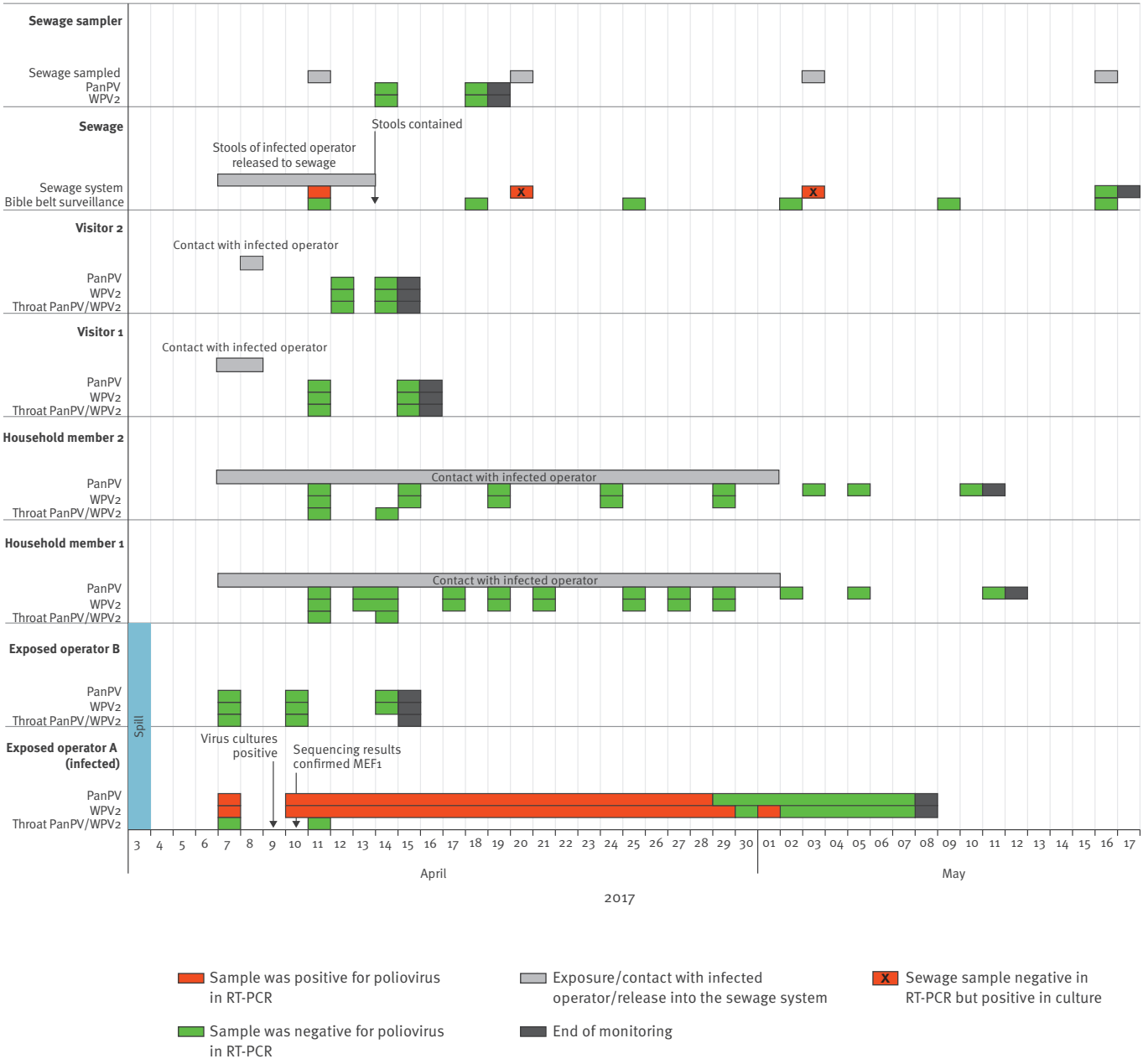
Environmental surveillance

During the first week after exposure, the toilet in the household of the infected operator was not disconnected from the sewage system and the WPV2 contaminated stool was thus not contained. To monitor WPV2 and its disappearance from the sewage system downstream of the residence, the system was sampled on 11 and 20 April and again on 3 and 16 May. WPV2 was detected by direct RT-PCR and by virus isolation in one of two sewage samples collected on 11 April. In two of five sewage samples collected on 20 April, and one of two samples collected on 3 May, WPV2 was detected only after virus isolation. Both samples collected on 16 May were negative for WPV2 and sewage monitoring downstream of the residence of the infected operator, was stopped.

The individual who collected the samples on 11 April was followed up by stool testing on day 4 and 7 after sampling: both stool samples were negative for poliovirus by RT-PCR and virus isolation. The individuals who collected later sewage samples were not followed up since WPV2 could not be detected in the concentrated samples by RT-PCR and it was either not detected or only after culture.

Figure

Timeline of the response, including sampling dates and poliovirus detections, to wild poliovirus type 2 (WPV2)-shedding event following accidental exposure to WPV2, the Netherlands, April 2017



Sewage sampler is the sampler of the sewage system downstream of the infected operator's residence.

Sewage system downstream of the infected operator's residence.

As a precautionary measure and to allow for early detection, the routine environmental surveillance applied in the regions with lower vaccination coverage (Bible belt) [4] was increased to weekly sampling from 11 April and was continued up to 16 May. So far, all samples from the routine environmental surveillance have tested negative for poliovirus.

Background

In 2015, WPV2 was officially declared eradicated by the World Health Assembly and the GAPIII was adopted [1]. Poliovirus facilities that serve critical international functions, including IPV and Sabin-IPV production, should manage biorisk appropriately, to minimise the risk of virus reintroduction into the environment and the community, and GAPIII requires very stringent containment of all PV2-related processes. From the PV2

eradication perspective, this is reasonable, however, it does not directly relate to a concrete public health threat, especially in countries with a strong hygienic infrastructure i.e. management of sewage waste water and a full IPV vaccination programme.

Discussion

We report on an infection with a WPV2 following a spill in a vaccine manufacturing plant. This is the first reported incident of its kind while stringent biorisk management systems in accordance with the GAPIII should be in place. Still, we identified gaps in the guidance for containment of WPV2 when an employee of such a plant excretes WPV2. The lessons learned will serve to update our national public health guidelines on poliovirus risk management.

Since the GAPIII document does not provide accurate descriptions for practical implementation of containment requirements, and the Dutch guidelines for polio were not GAPIII-compliant, we opted to combine a proportional public health response and several GAPIII requirements to deal with this public health incident.

The current follow-up protocol for possibly exposed employees of a facility requires the first sampling of stool and a throat swab on day 3/4 after the possible exposure. It aims at reliable exclusion of poliovirus infection and excretion based on the assumption that in the majority of cases, a spill does not result in an infection. Biorisk management procedures for WPV2 incidents in the post-eradication period, require early detection of poliovirus excretion. In order to achieve results timely, for WPV2 incidents, stool samples should be collected daily, starting immediately after the incident and be analysed by real-time RT-PCR. Even if direct detection by PCR may be less sensitive than virus isolation from cell cultures, the detection limits of current optimised protocols (<500 poliovirus RNA per gram stool) are such that virus loads below the detection limit for PCR are unlikely to pose a real risk for ongoing transmission.

Immediately upon the confirmation of WPV2 shedding by one of the operators, according to the Dutch procedures for outbreak control, an outbreak management team was convened. In our experience, multidisciplinary expertise is needed for a detailed risk assessment and effective incident management [8]. We focused in our risk assessment and containment measures on the faecal-oral transmission route, as the throat swabs of the infected operator were negative. These negative results are in agreement with the literature that shows that oral virus excretion by fully vaccinated persons is highly unlikely [9,10].

Initially, the WPV2-contaminated stools of the infected operator were not contained. The risk of poliovirus transmission through the sewage system was estimated to be very small since the sewage system concerned was a closed system with dilution and treatment at the sewage treatment plant (STP). Furthermore, it was checked and confirmed that there was hardly any contact between staff and potentially WPV2-contaminated sludge, the sewage sludge was incinerated and all workers of the STP were fully vaccinated against polio. The local public health services ensured that no sewage system maintenance works were in progress or planned in the near future downstream of the infected operator's residence up to the STP. One sewage pit downstream of the infected operator's residence remained positive for WPV2 for at least 20 days after disconnection from the sewage system, showing the need for containment of WPV2-positive stools. Disappearance of infectious virus from the local sewage system is the result of flushing of pipes, dilution and inactivation, and depends on factors such as number of households discharging to the system, constitution of waste discharged, precipitation, structure and integrity of the sewage system and temperature. As we show, and others suggested, this may take weeks to months (this study and [11]).

The containment of the stools of the infected operator was a challenge because a standard chemical toilet does not inactivate poliovirus and admittance to a hospital would have increased the risk of transmission of poliovirus to immunocompromised patients. Finally, we found a disposable system as used for Ebola virus disease patients to be useful and easily applicable at the home of the infected operator [7].

Balancing personal needs and the risk of infection, two family members of the infected operator remained in the same household throughout the isolation period. They were monitored and did not excrete poliovirus; they also agreed to comply with stringent hand and toilet hygiene and considerable restriction of their contacts and freedom to move outside the house. From a biorisk management perspective, this situation was far from ideal, however, isolation of the infected employee in a specially contained facility for more than 4 weeks would have severely disrupted family life.

Conclusions

Retrospectively, the measures undertaken to manage the public health risk were appropriate and proportional: during comprehensive monitoring no further spread of the virus was detected and there was acceptable impairment in the family life of the infected operator. The infected operator complied well with the stringent hygiene measures and prevented transmission of WPV2 to his household contacts. However, the containment of WPV2 in this event was not according to the biorisk management level expected in GAPIII. The GAPIII document is overall clear on what level of biorisk management for WPV2 is expected to prevent reintroduction of any PV2 into the environment and the community. It does, however, not provide practical guidance on how this should be achieved or on how to deal with an infected employee in the community (this report) or in a clinical/hospital setting [12].

The reported incident leading to WPV2 shedding by an exposed operator highlights that pre-GAPIII procedures and guidelines are insufficient in the post-GAPIII era. The biorisk management requirements as formulated in GAPIII need to be further translated into practical, probably country-specific, guidelines.

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Conflict of interest

None declared.

Authors' contributions

ED, WLMR, CPW and AT contributed to the conception and design of the study, data collection and analysis and writing of the article. All authors were involved in revising the manuscript and read and approved the final manuscript.

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Ongoing haemolytic uraemic syndrome (HUS) outbreak caused by sorbitol-fermenting (SF) Shiga toxin-producing *Escherichia coli* (STEC) O157, Germany, December 2016 to May 2017

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We report an ongoing, protracted and geographically dispersed outbreak of haemolytic uraemic syndrome (HUS) and gastroenteritis in Germany, involving 30 cases since December 2016. The outbreak was caused by the sorbitol-fermenting immotile variant of Shiga toxin-producing (STEC) *Escherichia coli* O157. Molecular typing revealed close relatedness between isolates from 14 cases. One HUS patient died. Results of a case–control study suggest packaged minced meat as the most likely food vehicle. Food safety investigations are ongoing.

In February 2017, five cases of haemolytic-uremic syndrome (HUS) were notified in Germany with onset of illness in week 5, 2017, which constituted a marked increase compared with the mean in the same week of the previous 5 years (mean: 0.6; range: 0–2 cases;). In parallel, the consultant laboratory (CL) for HUS at the University Hospital of Münster detected Shiga toxin 2-producing (*stx2*) sorbitol-fermenting (SF) *Escherichia coli* (STEC) O157:H- isolates in four HUS patients with disease onset between December 2016 and February 2017. We initiated an outbreak investigation to identify the cause of the outbreak, in order to control it.

Epidemiological investigation

SF *E. coli* O157:H-, *stx1*-gene negative, *stx2*-gene positive, *eae*-gene positive was identified as the outbreak strain. As at 22 May 2017, 30 cases, including one family cluster (n = 4), meet the outbreak case definition (Box): 14 confirmed, one probable and 15 possible cases have been reported. The outbreak is ongoing; the most recent symptom onset for a confirmed case was 13 April 2017 (Figure 1).

The 14 confirmed cases resided in the north-west of Germany and Berlin (Figure 2).

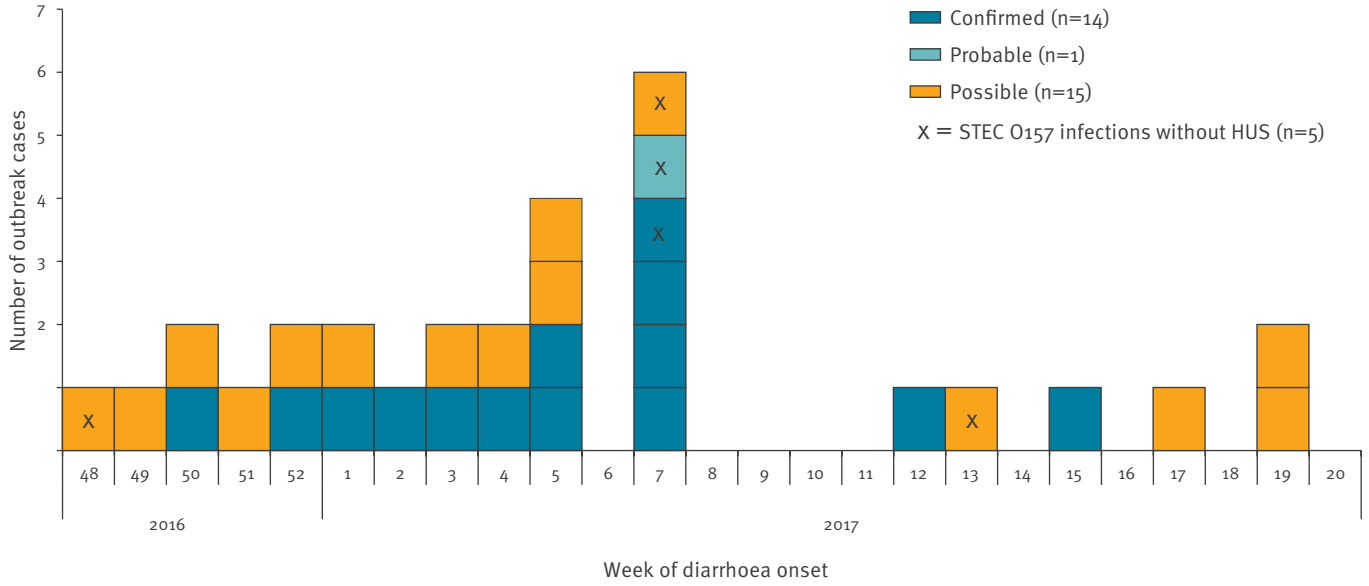
The mean age of the confirmed cases was 8.5 years (range: 1–36), half of them were male, 13 developed HUS and needed intensive care treatment; one patient died. None of the cases had a relevant pre-existing illness. The probable case, a middle-aged adult, a STEC enteritis case, was a close family member of three confirmed cases. Twelve of the 15 possible cases developed HUS and three had gastroenteritis; the mean age of the possible cases was 26 years (range: 0–83) and seven were male.

Explorative interviews

We interviewed 11 of the confirmed cases or their parents using a standardised trawling questionnaire containing questions on clinical symptoms, travel history, animal contacts, farm visits, other leisure activities and food consumption during 10 days before symptom onset.

Figure 1

Ongoing haemolytic uraemic syndrome and gastroenteritis outbreak caused by sorbitol-fermenting Shiga toxin-producing Escherichia coli O157, Germany, December 2016–May 2017 (n = 30 outbreak cases)



HUS: haemolytic uremic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

During 11 explorative interviews, we identified a number of frequently named food items, e.g. minced meat (beef and pork mixed), hot dog-style (Vienna) sausages, as well as several commercially available yoghurts or puddings. Cases and parents also reported having frequently shopped at supermarket chain X. They reported no common place of exposure. None of the cases had travelled in Germany or abroad. Few had had contact with a dog or cat (n = 4), one case had had contact with a donkey, a horse, a sheep, and a goat. One had consumed unpasteurised milk. None reported a farm visit or contact with cattle. This supported our initial hypothesis of a food-borne outbreak, which we had suspected based on biological plausibility and the wide geographical distribution of cases.

Case–control study

Following the interviews we conducted a case–control study to investigate the association of illness with food items named frequently in the explorative interviews. For each confirmed case under 13 years of age, we recruited three to six children reported to the local health authorities with another notifiable disease (e.g. influenza), who lived in the same or a neighbouring district as the outbreak case, and belonged to the same age group (1–4; 5–9; 10–12 years). Parents of cases and controls were interviewed by telephone after they had given informed consent. We used a short standardised questionnaire which covered the consumption of the above mentioned food items in the 10 days before symptom onset with details such as brand names, packaging, shopping places, etc.

We analysed the data using MS Excel and Stata 14.1 (Stata Corporation, Texas, United States), compared cases and controls by Chi-squared and Student t-test and estimated matched odds ratios (OR) for consumed food items and supermarkets using the Mantel-Haenszel test.

We included nine confirmed cases and 35 individually matched controls in the analysis. Cases and controls did not differ by sex and age; there was no association between being a case and having consumed different types of desserts (data not shown). Cases had eaten minced meat (beef and pork mixed) significantly more frequently than controls (Table). All cases (6/6) with the respective information reported this exposure. The parents of the cases prepared minced meat at home significantly more frequently than parents of the controls and they were significantly more likely to have purchased the minced meat at supermarket chain X (OR: 14.1; 95% confidence interval (CI): 1.2–174.9). However, only three of eight cases were explained by this exposure and the CI was wide. Shopping mostly or exclusively at supermarket chain X was associated with a threefold odds for being a case (OR: 3.0; 95% CI: 0.8–11.4).

Microbiological investigation

The microbiological investigations at the National Reference Centre for *Salmonella* and other Bacterial Enteric Pathogens at the Robert Koch Institute (RKI, national public health institute) and the CL for HUS included serotyping, testing for Shiga toxins, other phenotypic and genotypic markers and molecular

Box

Case definition, haemolytic uraemic syndrome outbreak caused by sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157, Germany, December 2016–May 2017

Outbreak cases were defined as cases of HUS[a] or STEC infection[b] with symptom onset starting on 1 December 2016, and residency in Germany.

Confirmed cases were defined as:

• cases of HUS or with STEC infection, with laboratory confirmation of the outbreak strain via WGS base typing or PFGE: SF *Escherichia coli* O157:H-; *stx1*-gene negative, *stx2*-gene positive, *eae*-gene positive.

Probable cases were defined as:

• cases of HUS with laboratory confirmation of SF STEC O157;

• cases of STEC infection with laboratory confirmation of SF O157;

• cases of HUS, STEC infection or bloody diarrhoea with an epidemiological link to a confirmed or probable case.

Possible cases were defined as:

• cases of HUS without any pathogen verification, or *E. coli* without confirmation of SF characteristic, or with confirmation of *E. coli* serogroup O157, excluding individuals with a laboratory test result conflicting with any characteristic of the outbreak strain, e.g. other serogroup.

• cases of STEC infection with *E. coli* serogroup O157 laboratory confirmation;

• cases of HUS, STEC infection or bloody diarrhoea with an epidemiological link to a possible case.

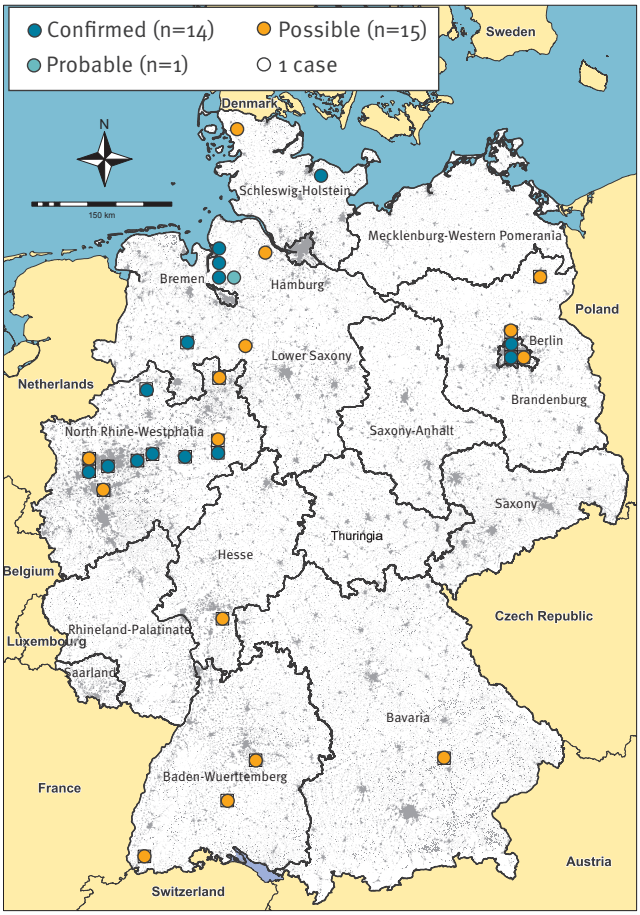
HUS: haemolytic uremic syndrome; PGFE: pulsed-field gel electrophoresis; SF: sorbitol fermenting; STEC: Shiga-toxin producing *Escherichia coli*; WGS: whole genome sequence.

[a] HUS is defined as at least two of the following three criteria: haemolytic anaemia, thrombocytopenia ≤ 15.000 cells/mm³ or renal impairment; or diagnosis of acute enteropathic HUS by a clinician, or death caused by HUS.

[b] STEC infection clinically defined.

Figure 2

Geographic distribution of cases, ongoing outbreak of haemolytic uraemic syndrome and gastroenteritis caused by sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157, Germany, December 2016–May 2017 (n = 30 outbreak cases)



The grey areas indicate densely populated regions.

subtyping (PFGE and WGS). We applied a threshold of › 10 alleles difference to securely exclude isolates from the outbreak [1]. Raw reads of the index case are available for download at http://www.ebi.ac.uk/ena under the study accession number PRJEB20962.

Molecular typing revealed close relatedness between isolates from 14 confirmed cases. The probable case had a PCR-positive stool sample sharing outbreak specific markers but culturing from stool was unsuccessful. Of the possible cases, all three STEC enteritis and four of the twelve HUS cases were positive for STEC O157, without any further typing. For the remaining eight HUS cases, isolates were not available.

Food safety investigations

State food safety authorities conducted inspections and sampling at minced meat producing plants supplying supermarket chain X and others. All results of official samples on production sites, retail level and from regional monitoring programmes have been negative for SF STEC O157 so far. Trace-back investigations starting from locations where cases had purchased minced meat or hot dog-style (Vienna) sausages are ongoing.

Discussion

We report an ongoing, protracted and geographically dispersed outbreak of HUS and gastroenteritis in Germany caused by SF STEC O157:H-. The supra-regional occurrence of cases makes a food item the most likely vehicle of transmission. Based on the investigations to date, we suspect packaged minced meat (beef and pork mixed) sold at one or several supermarket chains, as the most likely source. Other food items purchased at supermarket chains, in particular pre-packaged hot-dog style (Vienna) sausages, cannot be excluded as vehicle at this moment in time, but appear to be less plausible mainly for two reasons: less cases can be explained by this exposure compared to minced

Table

Association between being a case and having consumed certain food items, ongoing haemolytic uraemic syndrome and gastroenteritis outbreak caused by sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157, Germany, December 2016–May 2017 (n = 9 cases; n = 35 controls)

Variable	Exposed cases		Exposed controls		OR	95% CI	p value
Consumption of	**n**	**%**	**n**	**%**			
Heated minced meat	**6/7**	**86**	**19/33**	**58**	**5.5**	**0.6–52.9**	**0.096**
Heated mixed minced meat (beef and pork)	**6/6**	**100**	**9/23**	40	NC	NA	**0.015**
Minced meat bought from supermarket chain X	3/8	38	1/33	3	**14.1**	**1.2–174.9**	**0.007**
Minced meat bought from supermarket chain Y	2/7	29	6/35	17	2.0	0.4–10.4	0.424
Raw minced meat	1/8	13	5/34	15	2.1	0.09–49.2	0.637
Vienna sausages	6/8	75	8/34	35	6.3	0.6–63.6	0.073
Vienna sausages bought from supermarket chain X	4/6	67	5/12	42	2.3	0.2–28.3	0.493
Vienna sausages bought from supermarket chain Y	1/6	17	3/12	25	0.5	0.02–14.9	0.683
Preparation of dish with minced meat in the household (at least once a week versus less than once a week)	7/9	78	11/35	31	**8.6**	**1.2–60.6**	**0.009**
Shopping at supermarket chain X (always or mostly)	5/8	63	9/35	26	3.0	0.8–11.4	0.087

CI: Confidence interval; NA: not applicable; NC: not calculated; OR: odds ratio.
Significant results are displayed in bold.

meat and the sausages are heated during the production process and therefore contamination appears less likely.

SF STEC O157:H- was first described in Germany in 1988 [2] and subsequently caused several outbreaks in Germany [3-5] and elsewhere in Europe [6,7]. SF STEC O157 is characterised by a high pathogenicity [8,9]. One of the patients in the present outbreak died, and all 10 HUS cases whose parents were interviewed needed intensive care and dialysis. Few secondary gastroenteritis cases among family members were observed. Similarly to previously described outbreaks caused by SF STEC O157, this outbreak spanned several months and cases were geographically dispersed [4,5].

Little is known about the SF STEC O157 reservoir. In previous investigations, the pathogen was found in cattle and reindeer [10-12]. In other outbreaks, SF STEC O157:H- infections were epidemiologically linked to the consumption of mortadella (Italian-style sausage) and smoked pork paté [5] or to visiting one particular playground [3]. SF STEC O157 was isolated from minced beef products in an outbreak involving 18 HUS cases in France in 2011 [6]. In an outbreak associated with a recreational farm visit in Finland in 2012, unpasteurised milk was the most likely vehicle and isolates from patients' stool samples, cattle and the farm environment were identical [7].

At this point, investigations focus on minced meat as outbreak vehicle, although the available evidence is weak. The source of the contamination with SF STEC O157 is so far unknown. For minced meat, contaminations at the farm, slaughterhouse or subsequent production steps, are all possible options. It is thus conceivable that several retail outlets may be concerned.

One limitation in the investigation of this outbreak is that the number of interviewed confirmed cases is small and therefore, results of the case–control study should be interpreted with caution. For a number of HUS cases, no isolate was available and there was no opportunity of laboratory confirmation. In order to identify outbreak cases and allocate them appropriately, diagnostic laboratories and public health services are requested to send any STEC isolate from HUS patients to a typing centre. The use of genomic methods is desirable as routine for characterising all isolates.

A further limitation is that for a substantial proportion of cases, typing and interviews could not be conducted at all, or only weeks after symptom onset. Physicians, diagnostic laboratories, including national reference and consultant laboratories, public health and veterinary authorities at local, state and federal level, should be aware that timely actions are crucial for early detection of the vehicle and source.

Several previous investigations into outbreaks with SF STEC O157 have been unsuccessful in identifying the source, despite intensive efforts of health and food safety authorities [3-5]. This is the largest outbreak of SF STEC O157 in Germany since 2002 [4] and it has lasted for more than 4 months already. We assume that the source of infection may still be active and further cases may still occur. According to the feedback obtained via the Epidemic Intelligence Information System (EPIS) of the European Centre for Disease Prevention and Control (ECDC), the German outbreak

strain has not been detected in other European countries. In Germany, continued epidemiological investigations, sampling of food isolates and trace-back of food items are needed and ongoing to identify the cause of the outbreak.

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Conflict of interest

None declared.

Authors' contributions

Sabine Vygen-Bonnet, Bettina Rosner: leading investigators of the national outbreak investigation team at the RKI; interviews with cases and controls, data analysis and report writing.

Hendrik Wilking, Klaus Stark: scientific and coordination support, information exchange between federal and state authorities.

Juliane Seidel, Mirko Faber, Anika Schielke, Kai Michaelis, Alexandra Holzer: outbreak investigation team, i.e. communication with regional health authorities, laboratories and other stakeholders, interviews with cases and controls, data entry, regular updates of line list, epidemiological curve and map.

Daniela Kalhöfer, Sebastian Thole: outbreak investigation and interviewing of cases in the state of North Rhine-Westphalia.

Angelika Fruth, Rita Prager, Christina Lang, Sandra Simon, Antje Flieger: pathogen isolation, conduction and interpretation of molecular typing analysis of HUS and STEC enteritis patients' isolates, communication with diagnostic laboratories and public health services.

Annelene Kossow, Alexander Mellmann: diagnostics of stool samples, conduction and interpretation of molecular typing analysis of HUS and STEC enteritis patients' isolates, communication with clinicians, diagnostic laboratories and public health services.

Rolf Kamphausen: coordination of food safety interventions in North Rhine-Westphalia, organisation of information exchange between food safety authorities of federal states.

All authors have read and commented on the manuscript.

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Research article

Development and validation of the HCV-MOSAIC risk score to assist testing for acute hepatitis C virus (HCV) infection in HIV-infected men who have sex with men (MSM)

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Current guidelines recommend hepatitis C virus (HCV) testing for HIV-infected men who have sex with men (MSM) with ongoing risk behaviour, without specifying the type of risk behaviour. We developed and validated the HCV-MOSAIC risk score to assist HCV testing in HIV-infected MSM. The risk score consisted of six self-reported risk factors identified using multivariable logistic regression using data from the Dutch MOSAIC study (n = 213, 2009–2013). Area under the ROC curve (AUC), sensitivity, specificity, post-test-probability-of-disease and diagnostic gain were calculated. The risk score was validated in case–control studies from Belgium (n = 142, 2010–2013) and the United Kingdom (n = 190, 2003–2005) and in cross-sectional surveys at a Dutch sexually transmitted infections clinic (n = 284, 2007–2009). The AUC was 0.82; sensitivity 78.0% and specificity 78.6%. In the validation studies sensitivity ranged from 73.1% to 100% and specificity from 56.2% to 65.6%. The post-test-probability-of-disease ranged from 5.9% to 20.0% given acute HCV prevalence of 1.7% to 6.4%, yielding a diagnostic gain of 4.2% to 13.6%. The HCV-MOSAIC risk score can successfully identify HIV-infected MSM at risk for acute HCV infection. It could be a promising tool to improve HCV testing strategies in various settings.

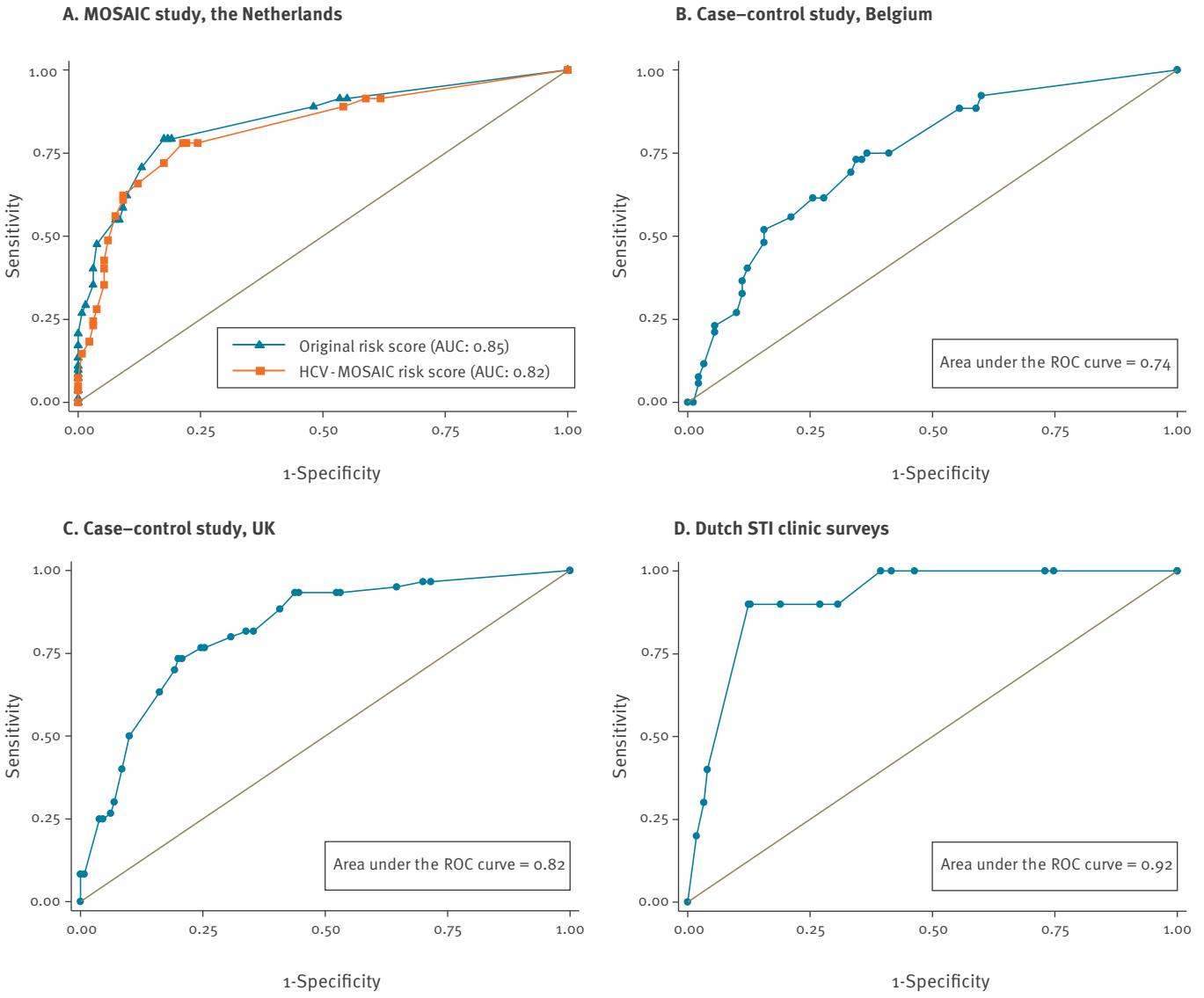
Introduction

Studies on hepatitis C virus (HCV) infections among HIV-infected men who have sex with men (MSM) have provided insights into the epidemiology and risk factors for sexually transmitted HCV acquisition [1,2]. As HCV transmission among MSM is ongoing in high-income countries worldwide [3,4], targeted testing is needed. Current national and international clinical guidelines recommend at least annual HCV antibodies (anti-HCV) testing for HIV-infected MSM who have unprotected (condomless) sex or who have been exposed to other, unspecified risk factors [5-7]. Furthermore, bi-annual alanine aminotransferase (ALT) testing is recommended for all HIV-infected patients [6,7]. In case of unexplained elevated ALT levels, subsequent HCV-RNA testing can be performed at the discretion of the physician. However, ALT is often not routinely measured in sexually transmitted infection (STI) clinics or other places outside of HIV care. Also, anti-HCV testing might not be sufficient in cases of an acute HCV infection as it takes several weeks or even months before anti-HCV can be detected in the presence of HIV [8,9]. Moreover, these guidelines include the presence of risk behaviour without specifying type and frequency.

Since early HCV detection and treatment may prevent onward transmission [10], more specific recommendations are required to identify who should be tested for acute HCV. A risk questionnaire could reduce the number of HCV tests performed in HIV-infected MSM, lowering costs and enhancing implementation of acute HCV testing in, for example, STI clinics. For chronic HCV infections, several risk scores or screening strategies to target those at highest risk for HCV were developed

Figure 1

Receiver operating characteristic curves for the original and HCV-MOSAIC risk score in the development study (A) and for the HCV-MOSAIC risk score in the three validation studies (B–D)



AUC: area under the ROC curve; HCV: hepatitis C virus; MOSAIC: MSM (men who have sex with men) Observational Study of Acute Infection with hepatitis C; ROC: receiver operating characteristic; STI: sexually transmitted infection; UK: United Kingdom.

[11-16]. However, to the best of our knowledge, risk scores identifying MSM at increased risk for acute HCV infection do not exist.

Recently, we examined risk factors for acute HCV infection in the MOSAIC study (MSM Observational Study of Acute Infection with hepatitis C). The MOSAIC study is an ongoing, prospective, observational cohort, enrolling HIV-infected MSM with acute HCV infection (cases) and one or two controls without a history of HCV for each case [17]. In this study we found that a high number (four or more) of risky sex acts was strongly associated with HCV acquisition [18]. Therefore, in the present study, we developed a risk score identifying at-risk MSM using data from this MOSAIC study and evaluated its sensitivity and specificity. In addition, we evaluated the performance of this risk score in three different populations of HIV-infected MSM, to assess whether this tool could be used to assist testing for acute HCV infection in HIV-infected MSM.

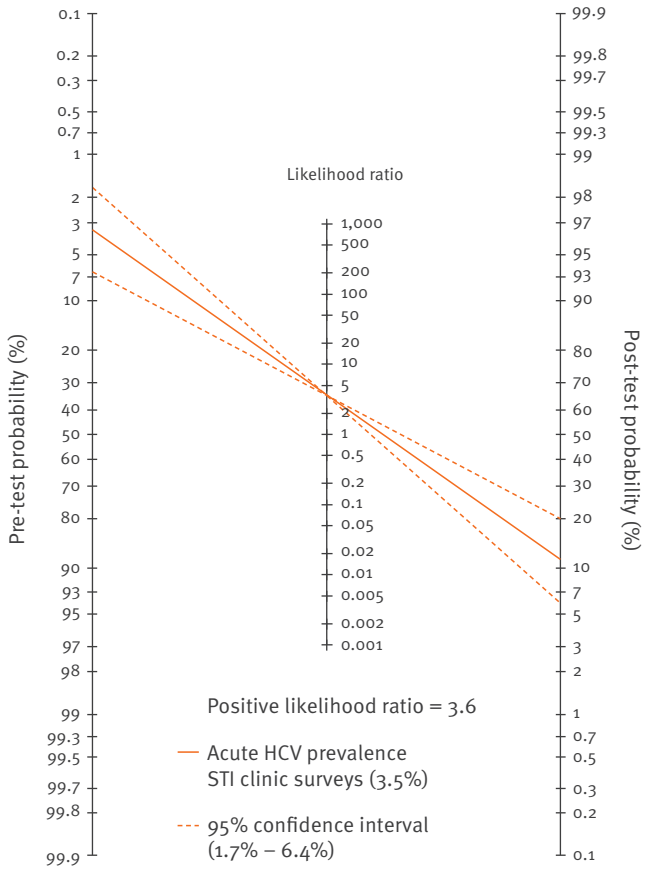
Methods

Development of the risk score

For the development of the risk score, all cases and controls enrolled in the MOSAIC study before February 2014 were selected. Acute HCV infection was defined as an interval≤6 months between the first positive HCV-RNA test and the preceding negative HCV-RNA or anti-HCV test. Information on risk factors for HCV was

Figure 2

Fagan's nomogram for a risk score of ≥ 2.0



HCV: hepatitis C virus; MOSAIC: MSM (Men who have sex with men) Observational Study of Acute Infection with hepatitis C; STI: sexually transmitted infection.

The Fagan's nomogram combines a range of pre-test probabilities of acute hepatitis C virus (HCV) infection (i.e. the prevalence range) with the likelihood ratio (LR) of the risk score, resulting in a range of post-test probabilities of acute HCV infection. It visualises diagnostic gain of the risk score (i.e. post-test probability minus pre-test probability).

obtained using a detailed self-administered questionnaire. Questions about risk behaviour refer to the 6 months preceding the moment of diagnosis with acute HCV for cases, and the 6 months preceding study entry for controls, except for questions about drug use and STIs, which refer to the past 12 months. The MOSAIC study was approved by the Institutional Review Board of the Academic Medical Center at the University of Amsterdam and ethical committees/board of directors of each institute recruiting participants; the assigned study numbers are NL26485.018.09 and NL48572.018.14.

For the development of the original risk score, we selected all risk factors that were statistically significantly associated with acute HCV in the multivariable logistic regression model including variables that potentially have direct effects on acquisition and variables that potentially facilitate transmission of acute HCV, as described elsewhere [18]. Subsequently, an individual risk score for each patient was calculated by summing the logistic regression beta-coefficients of all significant (p value‹0.05) risk factors reported.

Since the questions in the MOSAIC questionnaire are very detailed, we adjusted the original risk score to a revised risk score, which we will refer to as the HCV-MOSAIC risk score. For the HCV-MOSAIC risk score we used simplified definitions of the risk factors identified for the original risk score, making it suitable for validation and implementation. The HCV-MOSAIC risk score was constructed using the different beta coefficients derived from multivariable logistic regression analysis entering these simplified variables.

Validation of the risk score

We validated the HCV-MOSAIC risk score using three different study populations, for which we obtained the primary datasets. The first was a case–control study among HIV-infected MSM in care in three AIDS Reference Centers in Belgium from 2010 until 2013 [19]. Screening for anti-HCV was performed, followed by confirmation of positive samples by detection of HCV-RNA. All included participants had a negative anti-HCV test during the 12 months before their positive HCV test. For each case, the first two HIV-infected anti-HCV-negative MSM who visited the clinic after the case was included were selected as controls. The second was a case–control study in HIV clinics in the United Kingdom (UK) from 2003 until 2005 [20]. Cases were HIV-infected MSM with acute HCV infection, defined as a documented seroconversion to anti-HCV, accompanied by a positive HCV-RNA and/or clinical and biochemical criteria. The aim was to match two MSM controls without HCV for age, length of HIV infection, ethnicity and combination antiretroviral therapy (cART) exposure status. The third cohort was based on anonymous bi-annual cross-sectional surveys conducted at the STI clinic of the Public Health Service of Amsterdam in the Netherlands [21]. We used data collected between 2007 and 2009. Anti-HCV and HCV-RNA testing were performed in all HIV-infected MSM. Acute/recent HCV infection was defined as (i) HCV-RNA-positive and anti-HCV-negative or (ii) HCV-RNA-positive and anti-HCV-positive without a self-reported history of a previous positive HCV test. All other MSM with both a positive HCV-RNA and anti-HCV were excluded from the STI clinic dataset. The MSM who did not fulfil the criteria for acute/recent HCV infection were included in the analysis as HCV-negative.

Risk factors for HCV were collected at interview using a standardised questionnaire [19,21] or by a self-administered questionnaire [20]. Questions about risk behaviour referred to the 12 months before HCV diagnosis or study entry in the two case–control studies, and to the previous 12 months in the cross-sectional surveys.

Table 1

Characteristics of the development and three validation studies and their study populations and the variables of the HCV-MOSAIC risk score

Characteristics	Development study		Validation studies		
	MOSAIC study, the Netherlands (n = 213)		**Case–control study, Belgium (n = 142)**	**Case–control study, UK (n = 190)**	**Dutch STI clinic surveys (n = 284)**
Study design	Case–control		Case–control	Case–control	Cross-sectional
HCV status - HCV-positive (n) - HCV-negative (n)	 82[a] 131		 52 90	 60 130	 10 274
Study period	2009–2013		2010–2013	2003–2005	2007–2009
Median age in years (IQR)	45.7 (41.0–52.2)		45.0 (37.0–51.0)[b]	38.0 (33.5–41.9)[c]	42.0 (35.0–47.0)
Self-reported variables in the risk score	**HCV-MOSAIC risk score**	**beta**	**Deviations from the HCV-MOSAIC risk score**		
Condomless RAI 6M	Yes / no	1.1	RAI and condomless AI asked separately	ND	ND
Sharing of sex toys 6M	Yes / no	1.2	With casual sex partner(s)	ND	ND
Unprotected fisting 6M	Yes / no	0.9	ND	ND	ND
Injecting drug use 12M	Yes / no	1.4	During sex	ND	ND
Sharing of straws when NAD used 12M	Yes / no	1.0	ND	ND	Not asked
Ulcerative STI 12M	Yes / no	1.4	ND	Ever had syphilis or herpes	Not selfreported but tested

AI: anal intercourse; HCV: hepatitis C virus; IQR: interquartile range; MOSAIC: MSM (men who have sex with men) Observational Study of Acute Infection with hepatitis C; NAD: nasally administered drug; ND: no deviation; RAI: receptive anal intercourse; STI: sexually transmitted infection; ulcerative STI: syphilis, genital herpes or lymphogranuloma venereum infection; UK: United Kingdom; 6M: during the past 6 months; 12M: during the past 12 months.

[a] Nine reinfections.

[b] One missing value.

[c] Twenty seven missing values.

Statistical analysis

Using the MOSAIC data, the optimal cut-off point of the risk score to predict HCV positivity, defined as the highest sensitivity in combination with the highest specificity, was determined using Receiver Operating Characteristic (ROC) curves. The area under the curve (AUC) was calculated to assess accuracy of the risk score. Sensitivity and specificity with Wilson Score 95% confidence intervals (CI) were calculated for the optimal cut-off point. Differences between sensitivity and specificity from the development study and validation studies were evaluated using Newcombe's method 10 for independent proportions [22]. If the answer to a risk factor question was missing for a patient, we assumed that this risk factor was not present.

We could not reliably determine the positive and negative predictive value of the risk score, as these measures are dependent on the infection prevalence in the study group, and the case–control distribution in the development and validation studies, except for the Dutch STI clinic surveys, does not reflect the actual prevalence of acute HCV. To assess the clinical relevance, we calculated the post-test probability of HCV infection (i.e. the likelihood of being HCV-positive when given a positive HCV testing advice based on the risk score) using the formula [23]:

Formula 1

$$\frac{(\text{sensitivity} \times \text{prevalence})}{\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}$$

As the post-test probability of infection depends largely on the pre-test probability of infection (i.e. the prevalence of acute HCV infection in HIV-infected MSM, which we calculated using the data from the Dutch STI clinic surveys with its 95% CI as range), Fagan's

Table 2

Performance of the HCV-MOSAIC risk score among HIV-infected men who have sex with men in the development and three validation studies

	Development study	Validation studies		
	MOSAIC study, the Netherlands	Case–control study, Belgium	Case–control study, UK	Dutch STI clinic surveys
Sensitivity (95% CI)	78.0% (67.9–85.6)	73.1% (59.7–83.2)	93.3% (84.1–97.4)	100% (72.2–100)
Specificity (95% CI)	78.6% (70.8–84.8)	65.6% (55.3–74.6)	56.2% (47.6–64.4)	60.6% (54.7–66.2)
Proportion to be tested[a]	43%	49%	59%	42%
Area under the ROC curve (95% CI)	0.82 (0.76–0.88)	0.74 (0.66–0.83)	0.82 (0.76–0.88)	0.92 (0.85–0.98)

CI: confidence intervals; HCV: hepatitis C virus; MOSAIC: MSM (men who have sex with men) Observational Study of Acute Infection with hepatitis C; ROC: receiver operating characteristic; STI: sexually transmitted infection; UK: United Kingdom.

[a] Proportion of all cases and controls with a risk score of ≥ 2.0.

Table 3

Performance of the HCV-MOSAIC risk score for a range of different cut-offs among HIV-infected men who have sex with men in the development and three validation studies

	Development study		Validation studies					
	MOSAIC study, the Netherlands		Case–control study, Belgium		Case–control study, UK		Dutch STI clinic surveys	
Cutoff[a]	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
≥0.9	91.5	38.2	92.3	40.0	96.7	28.5	100.0	25.2
≥1.1	89.0	45.8	88.5	44.4	95.0	35.4	100.0	27.0
≥1.4	78.1	75.6	75.0	63.3	93.3	47.7	100.0	58.4
≥2.0	78.1	78.6	73.1	65.6	93.3	56.2	100.0	60.6
≥2.1	72.0	82.4	69.2	66.7	88.3	59.2	90.0	69.3
≥2.3	65.9	87.8	61.5	74.4	81.7	64.6	90.0	73.0
≥2.5	61.0	90.8	55.8	78.9	80.0	69.2	90.0	81.0
≥3.2	48.8	93.9	48.1	84.4	73.3	79.2	90.0	87.6
≥3.4	40.2	94.7	36.5	88.9	70.0	80.8	40.0	96.0
≥4.6	14.6	99.2	21.2	94.4	30.0	93.1	20.0	98.2

HCV: hepatitis C virus; MOSAIC: MSM (men who have sex with men) Observational Study of Acute Infection with hepatitis C; STI: sexually transmitted infection; UK: United Kingdom.

[a] Results are shown only for the cut-offs that were available in all four studies.

nomogram [24] was used to visualise the diagnostic gain (post-test probability minus pre-test probability of infection) after a positive testing advice. This graphical calculation of Bayes' theorem describes how positive testing advice changes the infection probability by combining the pre-test probability of acute HCV infection with the likelihood ratio (LR) of the risk score (which is calculated from sensitivity and specificity [23]), resulting in the post-test probability of acute HCV infection. All analyses were performed using Stata version 13.1 (Stata Statistical Software: Release 13; StataCorp LP, College Station, Texas, US).

Results

The MOSAIC development study enrolled 82 HIV-infected MSM with acute HCV and 131 HIV-infected MSM without a history of HCV as controls. The first validation study from Belgium included 52 cases and 90 controls and the second from the UK, 60 cases and 130 controls. Third, we included 10 HIV-infected MSM with acute HCV and 274 without HCV from the Dutch STI clinic surveys. Characteristics of the development and validation studies and their study populations are shown in Table 1; the median age of participants in all validation studies was significantly lower than the

median age in the development study (p value < 0.05 for all studies, Mann–Whitney U-test).

Development of the risk score

The previously described logistic regression model [18] identified the following six dichotomous risk factors for the original risk score: (i) condomless receptive anal intercourse (RAI) (beta 1.6); (ii) sharing of sex toys (beta 1.3) (both (i) and (ii) with HCV-positive or HCV-unknown sex partners); (iii) unprotected fisting (fisting without gloves, or with gloves but also group sex reported, beta 0.9); (iv) injecting drug use (IDU) during sex (beta 2.7); (v) sharing of straws when nasally administered drugs (NAD) used (beta 1.2); and (vi) self-reported ulcerative STI (syphilis, genital herpes or lymphogranuloma venereum infection, beta 1.6). Although statistically significant in the model, we excluded CD4 cell count, since its inclusion would make the risk score unusable in a setting where CD4 cell counts are not routinely measured (e.g. STI clinic). The best cut-off point for the original risk score, as determined using the ROC-curve (Figure 1A, AUC 0.85, 95% CI: 0.79–0.90) was ≥ 2.5. Sensitivity and specificity of the risk score using this cut-off point were 79.3% (95% CI: 69.3–86.6) and 82.4% (95% CI: 75.0–88.0) respectively.

For development of the HCV-MOSAIC risk score, as described in the methods we simplified the first four of the six risk factors, resulting in the following risk factors: (i) condomless RAI (with any partner, beta 1.1); (ii) sharing of sex toys (with any partner, beta 1.2); (iii) unprotected fisting (fisting without gloves, beta 0.9); (iv) IDU in the past 12 months (beta 1.4); (v) sharing of straws when NAD used (beta 1.0); and (vi) ulcerative STI (beta 1.4) (Table 1). The optimal cut-off point for the HCV-MOSAIC risk score became ≥ 2.0 and the ROC-curve had an AUC of 0.82 (95% CI: 0.76–0.88) (Figure 1A). When compared with the original risk score, the sensitivity of the HCV-MOSAIC risk score slightly dropped from 79.3% to 78.0% (95% CI: 67.9–85.6) and the specificity from 82.4% to 78.6% (95% CI: 70.8–84.8). The proportion of all participants with a risk score of ≥ 2.0 was 43% (92/213).

Validation of the risk score

The sensitivity and specificity of the HCV-MOSAIC risk score in the Belgian case–control study were 73.1% (95% CI: 59.7–83.2) and 65.6% (95% CI: 55.3–74.6), respectively. In the case–control study from the UK, sensitivity and specificity were 93.3% (95% CI: 84.1–97.4) and 56.2% (95% CI: 47.6–64.4), respectively. In the Dutch STI clinic surveys, sensitivity and specificity were 100% (95% CI: 72.2–100) and 60.6% (95% CI: 54.7–66.2), respectively (Table 2).

In the Belgian case–control study and the Dutch STI clinic surveys the sensitivity was lower and higher respectively than in the development study, but these differences were not statistically significant. In the study from the UK the sensitivity was significantly higher than in the development study (difference 15.3%, 95% CI: 3.3–26.2). Specificity was significantly lower in all validation studies compared with the development study (difference for the Belgian study 13.0%, 95% CI: 1.2–25.0, the UK study 22.4%, 95% CI: 11.1–33.0, and the Dutch study 18.0%, 95% CI: 8.5–26.6). The AUC in the validation studies ranged from 0.74 to 0.92 (Figure 1B–D). The proportion of participants (both cases and controls) with a risk score of ≥ 2.0 (i.e. the proportion of the population to be tested) in the validation studies ranged from 42% to 59% (Table 2). Table 3 shows the performance of the HCV-MOSAIC risk score for a variety of cut-offs in both the development and validation studies.

In the Dutch STI clinic surveys, data on one of the variables in the risk score (sharing of straws when NAD used) were not collected and therefore not scored. In a sensitivity analysis, we restricted the HCV-MOSAIC risk score in the development study to the same risk factors measured in the STI clinic (i.e. excluding sharing of straws): sensitivity decreased from 78.0% to 70.7% (95% CI: 60.1–79.5) and specificity increased from 78.6% to 83.2% (95% CI: 75.9–88.6).

Post-test probability

The post-test probability of acute HCV infection was calculated using the sensitivity and specificity of the HCV-MOSAIC risk score in the development study and using the prevalence of acute HCV in HIV-infected MSM in the Dutch STI clinic surveys, which was 3.5% (10/284 MSM, 95% CI: 1.7–6.4). The Fagan's nomogram (Figure 2) shows the post-test probability for a risk score of ≥ 2.0 and gives a precise overview of diagnostic gain.

The lines that start at the left y-axis show the HCV pre-test probability (i.e. 3.5%, range 1.7–6.4), cross the LR for a risk score of ≥ 2.0 (positive LR, i.e. sensitivity/(1–specificity)), then point to the HCV post-test probability at the right y-axis, which is 11.7% (range 5.9–20.0). The diagnostic gain of the risk score equals the difference between the infection probability for an individual before filling out the risk score (i.e. the prevalence) and the infection probability for an individual after being assigned to undergo HCV testing according to the risk score (i.e. HCV post-test probability). The diagnostic gain was 8.2% (11.7% minus 3.5%) and varied from 4.2% (5.9% minus 1.7%) to 13.6% (20.0% minus 6.4%).

Discussion

We developed and validated the first risk score for acute HCV infection in HIV-infected MSM. Using this risk score, 42–59% of HIV-infected MSM would be advised to undergo HCV testing, correctly identifying 73–100% of HIV-infected MSM with acute HCV infection, potentially making it a useful tool to assist testing for acute HCV infection. Our risk score could be implemented in settings where HIV-infected MSM are being tested for STIs, e.g. STI clinics. Currently, HCV testing is not routinely offered to MSM attending STI clinics in the Netherlands [25]. Moreover, the risk score could be

an addition to the current guidelines for HCV testing where risk behaviour as test criterion is not specified. Since all questions are self-reported, the development of a mobile-compatible website or application containing the risk score could be practical, ensuring confidentiality.

Although we consistently found >70% sensitivity, we need to emphasise that there is a proportion of HIV-infected MSM with acute HCV infection that will be missed when using the risk score. As described above this risk score should therefore be used as an additional tool rather than a replacement of testing practices in HIV clinics. Also, since the specificity was around 60% in the validation studies, a substantial proportion of HCV-negative MSM will be falsely identified as possible HCV-positive. However, since these MSM have a high score, our risk score could also be used to identify those who would benefit from interventions to reduce risk behaviour to prevent HCV infection.

Sensitivity and specificity of our risk score are within the higher range of those reported for existing risk scores to detect chronic HCV infection [11-16] and are also favourably comparable to existing risk scores to predict early HIV infection [26-28]. The diagnostic gain of the risk score ranged from 4.2% to 13.6%, which is slightly higher compared with the diagnostic gain of a risk assessment questionnaire for chronic HCV infection in the general population [14]. However, the diagnostic gain is dependent on the acute HCV prevalence in the population in which the risk score will be used and increases when prevalence is higher. A recent systematic review estimated a prevalence range of active HCV infection in HIV-infected MSM of 5.3–7.3% [29]. This range includes the upper limit of the prevalence we used (i.e. 6.4%). Use of our risk score will result in 42–59% of a population to be tested for HCV instead of everyone, which could potentially reduce test costs. However, cost-effectiveness studies are needed to compare different HCV testing strategies.

Our study has several limitations. First, there is heterogeneity between the development and validation studies. The performance of the risk score may have been influenced by differences in the definition of acute HCV between studies. We found 100% sensitivity in the Dutch STI clinic surveys, where it is likely that none of the acute HCV cases were missed because all men were simultaneously tested for HCV-RNA and anti-HCV. In addition, the questionnaires in the validation studies referred to risk behaviour in the last 12 months, whereas 3 of the 6 risk factors in our risk score refer to the last 6 months. The longer time period could have led to more risk behaviour acts reported, leading to a higher proportion with a risk score of ≥ 2.0. Also, study periods, countries and mode of questionnaire (at interview or self-administered) differed, and changes in risk behaviour over time or the social acceptability of some of the answers could have affected the performance of the risk score. Differences in HCV prevalence over time and between regions may have resulted in differences in the chance for an individual of being exposed to HCV, regardless of the level of risk behaviour. Second, we were unable to take into account the predictive value of an elevated ALT, since for the majority of the MOSAIC cases, HCV testing and diagnosis were based on an elevated ALT level. As current HCV testing practices in HIV treatment centres largely rely on the presence of an elevated ALT, the additional value of our risk score in combination with an elevated ALT can only be measured using a prospective validation study, as this would require testing for acute HCV in all patients with and without elevated ALT. We believe our risk score can be of added value, as ALT levels may remain within normal limits or rapidly normalise after acute HCV infection [30,31] and the sensitivity of an elevated ALT is reported to be as low as 20% for a recent HCV infection [31]. Third, our risk score was developed using data from a case–control study, while preferably a risk score should be developed using a prospective cohort study of HIV-infected MSM who are being regularly tested for acute HCV infection. A fourth limitation is that the sample sizes of the development and validation studies were relatively small.

Our risk score has not been validated among HIV-negative MSM, as their HCV prevalence is relatively low [21,32]. However, HCV infections have been reported in HIV-negative MSM using HIV pre-exposure prophylaxis [33-35]. For those people, it would be worth evaluating whether the risk score could assist HCV testing. Furthermore, our risk score was neither primarily developed nor validated for HCV reinfections. As reinfections are reported to be common in MSM [30,36,37], it could also be useful to validate the HCV-MOSAIC risk score in this group.

In conclusion, the HCV-MOSAIC risk score identifies HIV-infected MSM at risk for acute HCV infection. We encourage the use of this risk score, especially at testing locations where MSM are not regularly tested for HCV or where ALT is not routinely measured. It could be a valuable addition to the current guidelines for HCV testing and potentially reduce the amount of tests performed in MSM at low risk for acute HCV infection. In addition, it could be used as a tool to identify those who would benefit from interventions to reduce risk behaviour to prevent acute HCV infection.

MOSAIC collaborators

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Conflict of interest

MV served on a scientific advisory board for AbbVie, Bristol-Myers Squibb, Gilead Sciences, Johnson and Johnson, MSD and a data safety monitoring board for ViiV healthcare. Through his institution he received non-financial support by MSD. The remaining authors (AN, IS, JM, JS, JV, AB, MD, AH, MP) declared no conflict of interest.

Authors' contributions

All authors contributed significantly to the intellectual content of the manuscript. AN performed data analysis, and drafted the manuscript. IS and JV contributed to data management and analysis. JM and MV are physicians treating HIV-infected MSM, and together with JS, they also contributed to the intellectual content of the manuscript. AB and MP contributed to study concept and design. AB, MD and AH provided the data from the validation cohorts. MP is the principal investigator of the MOSAIC study.

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Research article

Human T-lymphotropic viruses (HTLV) in England and Wales, 2004 to 2013: testing and diagnoses

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Human T-lymphotropic virus (HTLV) infection has been under enhanced surveillance in England and Wales since 2002, however, little is known about testing patterns. Using data from two surveillance systems held at Public Health England, we described HTLV antibody testing patterns between 2008 and 2013 and the demographic and clinical characteristics of persons diagnosed with HTLV in England and Wales between 2004 and 2013. An increase in HTLV testing was observed in England between 2008 and 2013 (3,581 to 7,130). Most tests (82%; 7,597/9,302) occurred within secondary care, 0.5% (48/9,302) of persons were reactive for HTLV antibodies and 0.3% (27/9,302) were confirmed positive. Increasing age and female sex were predictors of a reactive HTLV screen and confirmed diagnosis. Testing in primary care including sexual health and antenatal services was infrequent. Between 2004 and 2013, 858 people were diagnosed with HTLV, most of whom were female (65%; 549/851), of black Caribbean ethnicity (60%), not born in the United Kingdom (72%; 369/514) and asymptomatic at diagnosis (45%; 267/595). Despite increased testing, the epidemiology and clinical features of those diagnosed with HTLV have remained consistent. Apart from donor screening, testing for HTLV infection remains uncommon, except to diagnose associated disease.

Introduction

The human T-lymphotropic viruses (HTLV), discovered in the early 1980s [1,2], have now infected an estimated 10 million people worldwide [3]. HTLV-1 is endemic in many tropical and subtropical regions, particularly the Caribbean, Iran, Melanesia, South America, southern India, southern Japan and West Africa [3]. In endemic areas, the distribution of infection varies, with seroprevalence among adults ranging between 0.1% and 30% [4]. In Europe and North America, HTLV-1 is predominately found among persons migrating from endemic areas, while HTLV-2 has been associated with injecting drug use [4].

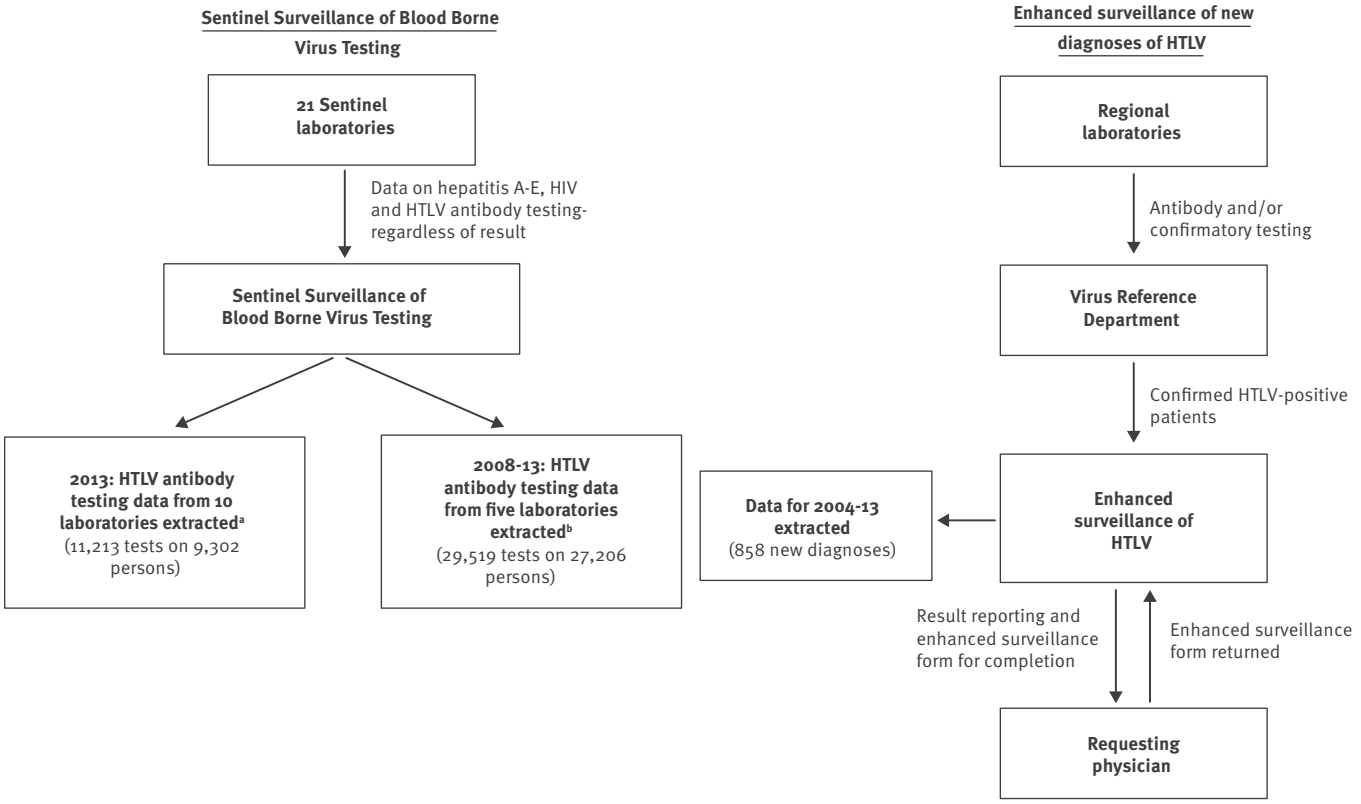
Although the majority (ca 90%) of HTLV-1-infected individuals remain asymptomatic carriers, the other 10% will develop one or more of several diseases [5]: 2–6% will develop adult T-cell leukaemia/lymphoma (ATLL), a highly aggressive T-cell malignancy, while 2–3% develop a variety of chronic inflammatory syndromes, most notably HTLV-1-associated myelopathy (HAM)/tropical spastic paraparesis (TSP) [6]. Other symptoms associated with HTLV infection include uveitis, thyroiditis, alveolitis, polymyositis and an impairment of immunity most strikingly associated with risk of strongyloidiasis observed in those HTLV-1 carriers exposed to *Strongyloides stercoralis*. There is no vaccine or effective treatment to reduce or eliminate HTLV viral load and treatments available for those who have malignant or inflammatory manifestations of HTLV infection are limited.

In endemic areas, HTLV is predominantly acquired through mother-to-child transmission (MTCT), primarily through breastfeeding [7], and through sexual contact, with male-to-female transmission approximately four times more likely to occur than female-to-male transmission [8]. However, there are no routine antenatal screening programmes in Europe. Blood transfusion has also been an important source of HTLV infection in some endemic areas, especially in Japan [4]. Blood, tissue and stem cell donations are currently routinely screened in a number of countries, including the United Kingdom (UK), while some blood services perform selective screening of first-time donors or rely on leucodepletion as an HTLV risk-reduction measure [9]. Other blood services, including many in Europe, have no screening programmes.

HTLV-1 testing was introduced in the UK in 1986 and routine testing of all blood donations was introduced in 2002 [10]. Surveillance by Public Health England (PHE; previously Health Protection Agency) was introduced in 1987 and enhanced surveillance in 2002 [11]. It is

Figure 1

Flow of data within the Sentinel Surveillance of Blood Borne Virus Testing, England, 2008–2013, and the enhanced surveillance of HTLV, England and Wales, 2004–2013



HIV: human immunodeficiency virus; HTLV: human T-lymphotropic virus.

[a] Laboratories offering testing in 2013.

[b] Laboratories testing for HTLV throughout the period 2008 to 2013.

estimated that more than 22,000 persons are infected with HTLV in England and Wales [12] and the seroprevalence in pregnant women in the UK is 0.3 per 1,000 [13]. However, there is little information on patterns of HTLV testing [14], which will influence the rate of undiagnosed HTLV. Here we examine the patterns of HTLV antibody testing in England from 2008 to 2013, with data from the Sentinel Surveillance of Blood Borne Virus Testing (SSBBV), along with HTLV diagnoses in England and Wales over the 10-year period between 2004 and 2013 collected through the enhanced surveillance of new diagnoses of HTLV.

Methods

Data sources

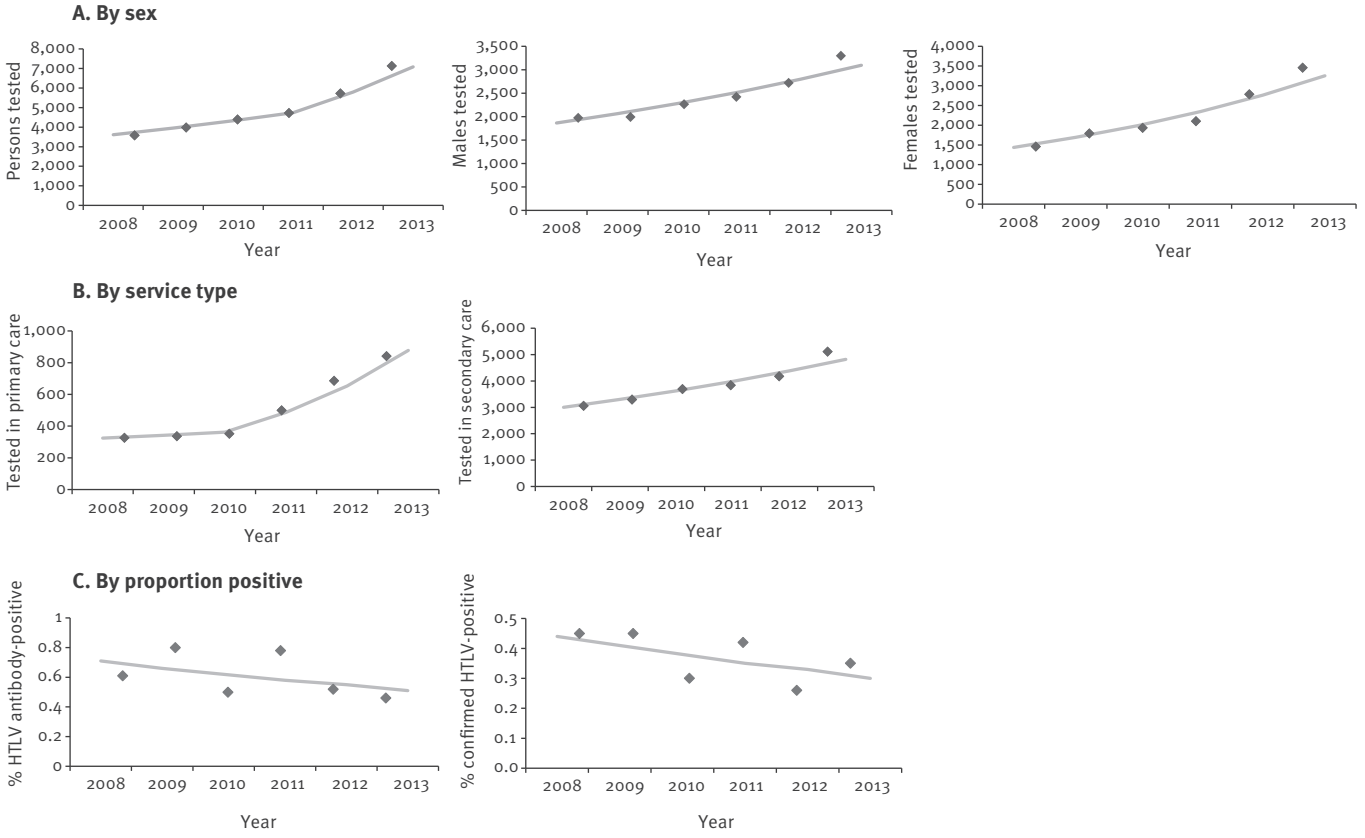
Two data sources were used for our analysis: (i) SSBBV, to look at HTLV antibody testing in 2013 and changes in testing between 2008 and 2013, and (ii) the enhanced surveillance of new diagnoses of HTLV to describe the epidemiological and clinical characteristics of persons diagnosed with HTLV between 2004 and 2013 (Figure 1).

SSBBV collects information on tests for hepatitis A–E, HIV and HTLV, regardless of result, from 21 participating sentinel laboratories in England. Individuals are de-duplicated and linked to all their test results, using a combination of coded surname (soundex), first initial, date of birth, National Health Service (NHS) number and clinic number [15]. Alongside the test result, SSBBV records demographic information and the service requesting the test. Information on HTLV antibody testing has been collected by SSBBV since 2005, with 10 laboratories offering HTLV antibody testing in 2013, five of whom were testing for HTLV throughout the period 2008 to 2013. Coverage varies annually, but in 2013, SSBBV captured front line testing for these viruses by laboratories covering ca 40% of the general population of England and is broadly representative of laboratories providing routine and reference testing.

Enhanced surveillance of new diagnoses of HTLV in England and Wales has been conducted since 2002 by PHE [12,16]. In brief, blood samples are sent to the Virus Reference Department (VRD) in PHE Colindale for either antibody and/or confirmatory testing, by Western blot

Figure 2

Trends in HTLV antibody testing in five sentinel laboratories, England, 2008–2013 (n = 29,519)



HTLV: human T-lymphotropic virus.

or PCR. Laboratory case reports of confirmed positive samples are provided to the HIV/sexually transmitted infection (STI) Surveillance Unit, who send an enhanced surveillance form to the referring clinician. The data collected include supplementary epidemiological and clinical information, including probable route of HTLV exposure, ethnicity, country of birth, probable country of infection and data on clinical symptoms at diagnosis. Enhanced forms are also received from NHS Blood and Transplant/PHE Epidemiology Unit for England and Wales when blood donors are found positive on routine donation screening. All data collected by PHE are pseudo-anonymised, with a soundex code and first initials collected instead of full names. Death reports on individuals who died from an HTLV-related illness are received from the Office of National Statistics and matched to the HTLV new diagnosis database using soundex code, date of birth and sex.

HTLV testing in 2013

Demographic and testing data for all individuals tested for HTLV antibodies in 2013 from 10 participating centres in England were extracted from SSBBV to give a snapshot of HTLV testing, as 2013 had the highest number of laboratories providing HTLV antibody tests for a full year. Where individuals had been tested more than once for HTLV antibodies, a reactive test was recorded over any negative or equivocal tests. Where all tests within 2013 were negative or equivocal, information from the most recent test was used.

Trends in HTLV testing: 2008 to 2013

To investigate trends, testing data for the period 2008–13 from the five sentinel laboratories with complete testing data for the 6-year period was used. Demographic and testing data for all individuals tested for HTLV antibodies between January 2008 and December 2013 were extracted from SSBBV. A cohort approach was used, with each individual recorded only once in each year tested. Where individuals had been tested more than once for HTLV antibodies in a year, a reactive test was recorded over any negative or equivocal tests. Where all tests in a year were negative or equivocal, information from the most recent test was used. Individuals who were reactive for HTLV antibodies were excluded from appearing in subsequent testing year cohorts.

HTLV testing in 2013 and trends in HTLV testing: 2008 to 2013

In both datasets, tests were linked to information on the location of the service requesting the test. Age at test was calculated using date of birth. Individuals younger than 1 year were excluded from the analysis, as

Table 1

Characteristics of persons testing for HTLV in 10 sentinel laboratories, England, 2013 (n = 9,302)

Patient characteristics	n	Reactive		Confirmed positive	
		n	%	n	%
Individuals	9,302	48	0.5	27	0.3
Sex					
Male	4,562	22	0.5	10	0.2
Female	4,312	26	0.6	17	0.4
Unknown	428	0	0.0	0	0.0
Age (years)					
1–29	1,901	8	0.4	2	0.1
30–44	2,528	8	0.3	2	0.1
45–59	2,480	22	0.9	16	0.6
≥ 60	2,094	10	0.5	7	0.3
Unknown	299	0	0.0	0	0.0
Service type					
Primary	862	4	0.5	4	0.5
Secondary	7,597	39	0.5	22	0.3
Other	843	5	0.6	1	0.1

HTLV: human T-lymphotropic virus

a reactive result at that age may reflect maternal HTLV antibody status. Individuals with a reactive HTLV antibody test were considered to have confirmed HTLV-1 or HTLV-2 infection if a Western blot or PCR performed at the VRD at PHE, which provides the confirmatory HTLV diagnostics, was positive. Requesting services were grouped into primary care (which included testing in general practice (GP), genitourinary medicine (GUM), accident and emergency (A and E), occupational health, prisons and drug dependency units) and secondary care services, along with an additional category called 'other', which included tests requested by blood and stem cell banks, research studies and where requester information was not available.

Enhanced surveillance of new HTLV diagnoses: 2004 to 2013

Data for the years 2004 to 2013 were used for these analyses, based on reports received by end of October 2015. Numbers may rise as further reports are received, particularly for recent years.

Statistical analysis

Unless otherwise stated, HTLV in this paper refers to both HTLV-1 and HTLV-2. Stata SE (Version 13.1) was used, with Wilcoxen rank-sum tests to compare continuous variables and chi-squared and Fisher's exact tests for categorical variables. A multivariate logistic regression, adjusted for age, sex, service type and year of test, was used to look at predictors of a reactive HTLV antibody test and confirmed HTLV diagnoses in those tested between 2008 and 2013. This dataset was used as more people were tested between 2008 and 2013 compared with the 2013 only dataset.

Joinpoint regression analysis was used to analyse changes in testing and positivity rates between 2008 and 2013, using Joinpoint Software (Version 4.3.1.0). Joinpoint identifies significant increases and decreases in testing and positivity rates over time, and whether there was a point at which a statistically significant change in the slope occurred during the period. The annual average percent change (APC) in testing numbers, with 95% confident intervals (95% CI), are reported. All proportions reported are excluding unknowns, and tests were considered statistically significant if the p value was lower than 0.05.

Results

HTLV testing in 2013

During 2013, 10 laboratories participating in sentinel surveillance reported HTLV testing in England, with 11,213 HTLV antibody tests conducted on 9,302 persons. Median age at testing was 45 years (interquartile range (IQR): 31–58 years), and men were older than women (49 vs 40 years; $p < 0.001$) (Table 1).

The majority (81.7%, 7,597/9,302) were tested within secondary care. Overall, the most common settings were haematology services (18.1%, 1,680/9,302), specialist renal services (13.4%, 1,250/9,302) and general medical or surgical departments (9.9%, 921/9,302). Among primary care settings, the most common for HTLV testing was GP with 265 tests (2.8% of all testing), followed by GUM services (2.7%, n = 247). In 2013, women's services requested just 62 tests (0.7%), comprising 22 HTLV tests within antenatal care, 12 in gynaecological services, 18 in birthing and obstetric services and 10 in postnatal services. During 2013, there were 664,517 live births in England [17].

Overall, 0.5% (n = 48) of persons tested were reactive for HTLV antibodies in 2013. HTLV antibody-reactive women were older than men (44 vs 55 years; $p < 0.001$), and the proportion of reactive tests was highest in persons aged 45–59 years. More than half (n = 27) of those reactive for HTLV were confirmed HTLV-positive by the VRD at PHE. The age and sex distribution of those with a confirmed HTLV-positive result was similar to those reactive to HTLV antibodies.

Trends in HTLV testing: 2008 to 2013

Five sentinel laboratories consistently reported HTLV testing each year between 2008 and 2013, with 29,519 HTLV antibody tests performed on 27,206 persons (13,316 in men and 12,558 in women, of those for whom sex was known). Overall, the number of persons tested increased by an average of 9.5% (95% CI: 7.4–11.7) annually between 2008 and 2011, and by 22.2% (95% CI: 17.5–27.2) annually between 2011 and 2013 (Figure 2 and Table 2).

The number tested each year between 2008 and 2013 increased by 17.8% (95% CI: 12.3–23.5) in women and by 10.7% (95% CI: 6.8–14.7) in men (Figure 2). Overall,

Table 2

Demographic characteristics and adjusted odds ratio of being reactive to HTLV antibodies and having a confirmed HTLV diagnosis, five participating sentinel centres, England, 2008–2013 (n = 29,519)

Patient characteristics	All tested (n = 29,519)	Reactive HTLV screen				Confirmed HTLV diagnosis			
		Number (n = 176)	aOR	95% CI	p value	Number (n = 89)	aOR	95% CI	p value
Age (aOR/10 year increase)	*45 (33–59)* [a]	*52 (40–63)* [a]	*1.2*	*1.1–1.3*	*0.001*	*53 (42–64)* [a]	*1.4*	*1.2–1.5*	*<0.001*
Sex									
Male	14,653	73	1	Ref		30	1	Ref	
Female	13,524	102	1.7	1.2–2.3	0.001	59	2.4	1.6–3.8	<0.001
Not reported[b]	1,342	1	NA			0	NA		
Service type									
Primary	3,043	15	1	Ref		12	1	Ref	
Secondary	23,142	152	1.2	0.7–2.0	0.6	75	0.6	0.3–1.2	0.1
Other	3,334	9	0.5	0.2–1.1	0.1	2	0.1	0.03–0.5	0.006
Year tested									
2008	3,581	22	1	Ref		15	1	Ref	
2009	3,977	32	1.3	0.8–2.3	0.3	14	0.9	0.4–1.9	0.8
2010	4,393	22	0.8	0.4–1.5	0.5	9	0.5	0.2–1.1	0.1
2011	4,719	37	1.3	0.8–2.2	0.3	16	0.9	0.4–1.8	0.7
2012	5,719	30	0.9	0.5–1.6	0.7	14	0.6	0.3–1.3	0.2
2013	7,130	33	0.8	0.5–1.4	0.5	21	0.8	0.4–1.6	0.5

aOR: adjusted odds ratio; CI: confidence interval; HTLV: human T-lymphotropic virus; NA: not applicable; Ref: reference value.

[a] Median and interquartile ranges.

[b] Persons of unknown sex were not included in the logistic regression.

the median age at test decreased from 47 years in 2008 to 43 years in 2013 ($p<0.001$) and from 44 years to 38 years among women ($p<0.001$); there was no significant change for men. While the number of persons screened for HTLV infection in primary care settings increased 157% overall, a result of more testing requested within GP and occupational health services, the APC was not significant (2008–10: 5.6%, 95% CI: −53.8 to 141 and 2010–13: 34.4%, 95% CI: −11.1 to 103). The number of people tested within secondary care increased 9.9% (95% CI: 6.7 to 13.2) annually between 2008 and 2013.

In the five sentinel laboratories, 0.6% of persons tested (n = 176) had a reactive HTLV antibody result between 2008 and 2013, with no significant difference in the proportion positive by year ($p=0.2$). All other variables remained stable. The specialities that found the most number of persons reactive for HTLV were haematology (n = 53) and general medical and surgical wards (n = 20). Among those with an initially reactive HTLV antibody result, 50.6% (n = 89) were subsequently confirmed to have HTLV, a prevalence of 0.3%.

Predictors of a reactive HTLV screen and confirmed HTLV diagnosis

Using a multiple logistic regression (Table 2), the odds of testing reactive for HTLV antibodies did not change by year or between primary and secondary care, but increased for each 10-year increase in age (odds ratio (OR) = 1.2; 95% CI: 1.1–1.3) and for women compared with men (OR = 1.7; 95% CI: 1.2–2.3). These findings were similar for a confirmed HTLV diagnosis (Table 2), although the odds of being confirmed following a test in 'other services' (i.e. not specified) were lower than in primary care (OR = 0.1; 95% CI: 0.03–0.5).

HTLV diagnoses in England from enhanced surveillance 2004 to 2013

Between 2004 and 2013, 858 new HTLV diagnoses were reported to PHE in England and Wales, averaging 86 cases per year (range: 76–98) (Table 3).

Where reported, more women were diagnosed with HTLV than men (64.5%; 549/851). Median age at diagnosis was 52 years (IQR: 42–66), with no difference between men and women (50 vs 53 years; $p=0.2$). The majority of diagnoses were among persons of black Caribbean ethnicity (59.7%, 328/549), followed by white (18.6%, n = 102), black African (12.0%, n = 66), other/mixed (4.9%, n = 27), south Asian (3.5%, n = 19) and black other ethnicities (1.3%, n = 7). When compared with white individuals, persons of other/mixed and South Asian ethnicity were younger (49 vs 36 and 29 years, respectively; both $p<0.05$), whereas persons of black Caribbean ethnicity were older (49 vs 53 years; $p<0.002$). Where country of birth was reported (59.9%, n = 514), the majority of HTLV-positive persons of black African (51/56), black Caribbean (231/284), South

Table 3

Demographic and clinical characteristics of persons reported to the enhanced surveillance of HTLV programme, England and Wales, 2004–2013 (n = 858)

Patient characteristics	n	%
Individuals	858	100.0
Sex		
Male	302	35.2
Female	549	64.0
Unknown	7	0.8
Age (years)		
1–29	81	9.4
30–44	183	21.3
45–59	308	35.9
≥ 60	284	33.1
Unknown	2	0.2
Ethnicity		
White	102	11.9
Black Caribbean	328	38.2
Black African	66	7.7
Black other	7	0.8
Indian/Pakistani/Bangladeshi	19	2.2
Other/mixed	27	3.1
Unknown	309	36.0
HTLV type		
HTLV-1	773	90.1
HTLV-2	40	4.7
Unknown	45	5.2
Exposure		
Heterosexual sex and/or mother-to-child transmission	365	42.5
Other [a]	36	4.2
Unknown	457	53.3
HTLV-associated symptoms		
Asymptomatic	267	31.1
HTLV symptoms	193	22.5
Non-HTLV symptoms	135	15.7
Unknown	263	30.7
Year of diagnosis		
2004–08	406	47.3
2009–13	452	52.7
HIV co-infection	43	5.0
Deaths	96	11.2

HIV: human immunodeficiency virus; HTLV: human T-lymphotropic virus.

[a] Includes transfused blood, sex between men and people who inject drugs.

Asian (14/17) and other/mixed ethnicity (17/25) were born abroad. Overall, 5.0% of persons diagnosed with HTLV (n = 43) were co-infected with HIV.

The majority of HTLV infections diagnosed, where type was reported, were HTLV-1 (95.1%, 773/813) and only 4.9% (n = 40) of persons were infected with HTLV-2. Most persons diagnosed with HTLV-1 were of black Caribbean origin (62.8%, 319/508 with known ethnicity), whereas most persons with HTLV-2 were of white ethnicity (n = 18).

Where reported (62.5%, n = 536), the most common reason for testing for HTLV was clinical symptoms (43.7%, n = 234), followed by blood donation (27.1%, n = 145). Persons diagnosed following blood donation were younger than those tested because of symptoms (41 vs 56 years; $p<0.001$) and a higher proportion of women tested positive in the context of blood donation than men (30.7%, 109/355 v 20.2%, 36/178; $p=0.01$). There was no difference by sex in the proportion of persons presenting with symptoms (41.7%, 148/355 vs 47.8%, 85/178; $p=0.1$). Persons of black ethnicity (54.9%, 200/364) and those diagnosed with HTLV-1 (45.6%, 226/496) were most commonly diagnosed following the presentation of symptoms, whereas persons of white (46/98), other/mixed (13/23) and South Asian ethnicity (14/19) and those with HTLV-2 (10/19) were most commonly diagnosed when tested in the context of blood donation.

Probable route of infection was reported for 46.7% (n = 401) of persons between 2004 and 2013. Among those, just over a quarter were probably infected through heterosexual sex (27.7%, n = 111), another quarter (25.2%, n = 101) via MTCT, and 38.2% (n = 153) reported both MTCT and heterosexual contact. MTCT and heterosexual contact were the most common routes of probable infection regardless of ethnicity or sex, but men were more likely to report other transmission routes than women (16.1% vs 5.3%; $p<0.001$) and almost all (9/10) persons with a probable infection following injecting drug use were of white ethnicity. A smaller proportion of persons infected with HTLV-2 reported a probable infection route of heterosexual contact and/or MTCT than those with HTLV-1 (57.9% vs 92.6%; $p<0.001$), with injecting drug use accounted for the remaining infection in HTLV-2 (n = 8).

The majority of persons with symptoms reported were asymptomatic (44.9%, 267/595), 20.5% (n = 122) had ATLL, 15.0% (n = 89) had other or non-HTLV related symptoms, 11.9% (n = 71) had TSP/HAM and 7.7 (n = 46) had other malignancies/neurology (Table 4).

Persons who were asymptomatic at diagnosis were younger than persons with ATLL and TSP/HAM (asymptomatic: 44 years vs ATLL: 55 years and TSP/HAM: 53 years, both $p<0.001$). Regardless of ethnicity, most persons were asymptomatic at diagnosis, but almost all persons with ATLL were of black ethnicity (90/94). Where reported, a higher proportion of persons with HTLV-2 were asymptomatic than with HTLV-1 (69.6% vs 44.0%; $p=0.02$).

Of all persons diagnosed between 2004 and 2013 in England and Wales, 96 (11.2%) are known to have died from HTLV-related illness. Deaths most commonly occurred in women (n = 57), persons of black Caribbean

Table 4

Demographic and clinical characteristics of persons reported to the enhanced surveillance of HTLV programme, by symptoms, England and Wales, 2004–2013

Patient characteristics	Asymptomatic		ATLL		TSP/HAM		Other HTLV symptoms		Non-HTLV symptoms		Not reported	
	n	%	n	%	n	%	n	%	n	%	n	%
Individuals	267		122		71		97		38		263	
Median age (IQR)	44 (33–54)		59 (50–69)		53 (46–68)		60 (46–73)		57 (47–72)		55 (43–68)	
Sex												
Male	85	31.8	42	34.4	19	26.8	40	41.2	17	44.7	99	37.6
Female	181	67.8	79	64.8	52	73.2	57	58.8	21	55.3	159	60.5
Not Reported	1	0.4	1	0.8	0	0.0	0	0.0	0	0.0	5	1.9
Ethnicity												
White	74	27.7	3	2.5	7	9.9	5	5.2	5	13.2	8	3.0
Black Caribbean	117	43.8	77	63.1	44	62.0	45	46.4	23	60.5	22	8.4
Black African	31	11.6	10	8.2	10	14.1	8	8.2	4	10.5	3	1.1
Black Other	3	1.1	3	2.5	0	0.0	1	1.0	0	0.0	0	0.0
Indian/Pakistani/Bangladeshi	16	6.0	0	0.0	1	1.4	1	1.0	0	0.0	1	0.4
Other/Mixed	18	6.7	1	0.8	0	0.0	2	2.1	2	5.3	4	1.5
Not Reported	8	3.0	28	23.0	9	12.7	35	36.1	4	10.5	225	85.6
Exposure												
Heterosexual sex and/or mother to child transmission	189	70.8	54	44.3	45	63.4	38	39.2	25	65.8	14	5.3
Other [a]	26	9.7	1	0.8	1	1.4	4	4.1	2	5.3	2	0.8
Not Reported	52	19.5	67	54.9	25	35.2	55	56.7	11	28.9	247	93.9
HTLV type												
HTLV-I	241	90.3	120	98.4	68	95.8	87	89.7	32	84.2	225	85.6
HTLV-II	16	6.0	0	0.0	0	0.0	4	4.1	3	7.9	17	6.5
Unknown	10	3.7	2	1.6	3	4.2	6	6.2	3	7.9	21	8.0
HIV Co-infection	16	6.0	5	4.1	2	2.8	8	8.2	6	15.8	6	2.3
Deaths	4	1.5	53	43.4	3	4.2	17	17.5	4	10.5	15	6.7

ATLL: T-cell leukaemia/lymphoma; HAM/TSP: HTLV-1-associated myelopathy/tropical spastic paraparesis; HTLV: human T-lymphotropic virus.
[a] Includes blood transfusion, sex between men and people who inject drugs

ethnicity (n = 47), persons with HTLV-1 (n = 89) and persons with ATLL (n = 53). Median time between HTLV diagnosis and death was 156 days (IQR: 28.5–324.5).

Discussion

An estimated 22,000 persons are infected with HTLV in England and Wales, yet each year an average of only 86 HTLV diagnoses are made. To explore why there is this discrepancy, we investigated HTLV testing patterns from sentinel laboratories in England. The numbers of persons tested for HTLV increased between 2008 and 2013, and the majority of HTLV tests occurred in secondary care. The biggest proportional increases in testing were seen among women and in primary care services but from a very small baseline, and testing in these settings remains uncommon. Although more testing occurred in men, women are more likely to have a reactive HTLV test and confirmed HTLV diagnosis.

This paper also updates the epidemiological picture of HTLV provided through enhanced surveillance since it was last described in 2002. The number of persons being diagnosed with HTLV remained constant over the 10-year period. The majority of persons diagnosed were female, of black Caribbean ethnicity and were born outside the UK. HTLV-1 remains the most common form of HTLV diagnosed in England and Wales and most patients were asymptomatic at diagnosis and infected through either heterosexual contact or MTCT. In contrast, the smaller fraction diagnosed with HTLV-2 were more likely to be white, asymptomatic and have a history of injecting drug use.

Our results collected through the enhanced surveillance are consistent with previous descriptions of HTLV-diagnosed persons in England and Wales [12,16], with similar risk factors, particularly by subtype. Compared with Spain, which has conducted similar surveillance, more cases were diagnosed per annum in England and Wales (89 vs 25) [18]. In addition, a higher proportion of cases in England and Wales had HTLV-1 when compared with Spain (90.1% vs 26.1%) and were linked to ATLL (20.5% vs 7.3%), whereas similar proportions were

linked to HAM/TSP (England and Wales: 11.9%, Spain: 10.9%).

The vast majority of studies worldwide present data through blood donor testing, antenatal screening and enhanced surveillance, with limited information on general testing patterns. Sentinel surveillance of blood-borne virus testing presents a unique opportunity to gain an insight into where HTLV tests are being requested in England and reviewing changes in testing and prevalence estimates.

Like HIV and hepatitis, HTLV has a long latency period, and a person can be infected for many years before receiving a diagnosis or presenting symptoms. Therefore, it is likely that the vast majority of infections in England and Wales, estimated at 22,000 persons, remain undiagnosed [12]. The introduction of routine HTLV screening of tissue, blood and stem cell donations in 2002, alongside increased disease awareness, may have driven some of the observed increase in HTLV testing. However, the majority of tests still occurred in secondary care specialities where symptomatic patients are more likely to present or where blood, tissue and stem cell donation is common (haematology, renal services and ophthalmology). Therefore, these testing practices are unlikely to impact the undiagnosed proportion. Nevertheless, increased disease awareness, facilitated by the expansion of specialist HTLV clinics from one to four sites in England (London, Birmingham, Manchester and York), will ensure that testing for HTLV will be more widely considered outside of traditional settings.

Our testing data suggests potential missed opportunities for the prevention of maternal transmission of HTLV. While we observed some testing in antenatal, birthing and post-natal units, it is likely that much of this testing is being driven by the screening of tissue for storage and donation, rather than prevention of HTLV transmission, and primary care testing remains infrequent, implying that testing for HTLV infection is uncommon within sexual health services in England.

Prevalence of HTLV in some groups, particularly older individuals and women, was high. Higher positivity rates in older persons are likely to be due to low disease awareness and the long latency period where a person will remain asymptomatic before developing symptoms and being tested. Higher positivity rates in women are probably due to a higher prevalence in women overall, which may result from differences in the efficiency of HTLV transmission between male and female sexual partners, with transmission rates higher from HTLV-positive male partners [8,19]. Work to understand transmission within family settings in Japan estimated transmission rates from men to women to be as high as 60.8%, compared with 0.4% in female-to-male transmission [20], with similar outcomes in studies conducted in Jamaica and the United States [21,22]. In the UK, there are currently no seroprevalence studies with sufficient statistical power to assess differences by sex, however, the prevalence of HTLV in blood donors is higher in women [23]. In addition, these higher positivity rates may reflect differences in the presentation of females to care, with women more likely to present at healthcare settings earlier than men [24-26]. Lima et al. indicated that women in Brazil had faster disease progression than men [27].

The high rate of confirmation of HTLV infection following a reactive result in the initial test supports the high specificity of the currently available assays. This is important as there is concern, particularly in low prevalence regions, that even minimal lack of specificity places a large burden on diagnostic laboratories to investigate many false-negative results. Our finding that more than half of reactive antibody tests were confirmed positive is thus reassuring.

Although 72% of HTLV infections occurred in persons of black Caribbean or black African ethnicity, 94% of diagnoses of ATLL occurred within these ethnic groups. The Caribbean as well as western and southern Africa are endemic regions for HTLV infection and thus there is greater potential for mother-to-child HTLV transmission. HTLV-1 infection acquired perinatally or in infancy through breast-feeding has been associated with risk of ATLL, whereas HTLV-1 infection acquired in England and Wales is more likely to have been due to behaviour during adulthood. These data therefore support the assumption of ATLL risk and also draw attention to the need to consider HTLV-1 infection in any patient with a T-cell malignancy, but especially if they belong to a high-risk ethnic group.

There are two key limitations in our study. The first is the small number of persons with a positive HTLV screen and confirmed HTLV diagnosis. Despite our testing data indicating an increase in testing, with similar proportions testing positive for HTLV, we have not seen the number of persons reported to the enhanced surveillance of HTLV increase over the same time period. In addition, the small number of people diagnosed with HTLV prevented us from looking at demographic and clinical trends. Secondly, we were unable to look at trends in ethnicity in HTLV testing through the SSBBV testing database. It would have been particularly interesting to see whether particular groups are being targeted for testing in line with demographics, with persons of black Caribbean ethnicity representing the majority of those diagnosed with HTLV in England and Wales. Despite these limitations, enhanced surveillance of HTLV has high case ascertainment of persons diagnosed with HTLV in England and Wales, as all confirmatory tests are directed to PHEs VRD, ensuring that our sample is representative.

Since 2013, there have been significant changes to the surveillance of new diagnoses of HTLV, which has resulted in key clinical information being underreported (‹30% completion rates), making comparison

with more recent data problematic. Work is ongoing to improve the completeness of the dataset. However, we do not expect the characteristics of persons newly diagnosed with HTLV between 2014 and 2015 to differ significantly from those diagnosed between 2004 and 2013.

Although characteristics of those with a confirmed diagnosis of HTLV reported to the enhanced surveillance in England and Wales do not appear to have changed over the past 10 years, HTLV testing has increased in England. This increase occurred predominately through increased awareness of HTLV, associated with symptom presentation. Although there have been increases in testing, testing frequency in primary care services, including sexual health services, is still suboptimal and continued work is needed to ensure that HTLV is considered among those at greater risk, tackling the undiagnosed fraction and reducing transmission.

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Conflict of interest

None declared.

Authors' contributions

GT conceived the paper. KD, SC and PH developed the idea with GT. JT, SC and RR collected and processed the enhanced surveillance of HTLV data. GI extracted data from the sentinel surveillance of blood-borne virus testing, matched the two datasets and undertook the data analysis of both datasets, RS and GT supervised. GI drafted the paper and all authors provided critical input to the manuscript and approved all revisions.

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