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Recent and rapid transmission of HIV among people who inject drugs in Scotland revealed through phylogenetic analysis

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Short summary: An outbreak of HIV among people who inject drugs in Scotland follows similar recent outbreaks in Greece, Romania, Ireland and the USA. Phylodynamic analysis demonstrates the infections are tightly linked genetically and the effective reproductive number remains above 1.

1 ABSTRACT

Harm reduction has dramatically reduced HIV incidence among people who inject drugs (PWID). In Glasgow, Scotland, <10 infections/year have been diagnosed among PWID since the mid-90s. However, in 2015 a sharp rise in diagnoses was noted among PWID: many were subtype C with two identical drug resistant mutations and some displayed low avidity, suggesting the infections were linked and recent.

We collected Scottish *pol* sequences and identified closely related sequences from public databases. Genetic linkage was ascertained among 228 Scottish, 1820 UK and 524 global sequences. The outbreak cluster was extracted to estimate epidemic parameters.

All 104 outbreak sequences originated from Scotland and contained E138A and V179E. Mean genetic distance was <1% and mean time between transmissions was 6.7 months. The average number of onward transmissions consistently exceeded 1, indicating that spread was ongoing.

In contrast to other recent HIV outbreaks among PWID, harm reduction services were not clearly reduced in Scotland. Nonetheless, the high proportion of individuals with a history of homelessness (45%) suggests that services were inadequate for those in precarious living situations. The high prevalence of Hepatitis C (>90%) is indicative of sharing of injecting equipment.

Monitoring the epidemic phylogenetically in real-time may accelerate public health action.

2 INTRODUCTION

People who inject drugs (PWID) are at risk of acquiring HIV from sharing injecting equipment and from high risk sexual activity while under the influence of drugs or in exchange for drugs[1]. There are 9-22 million PWID worldwide of whom 1-5 million have HIV [2].

Major outbreaks of HIV were identified among PWID in Scotland in the 1980s [3-5], along with other parts of northern [3, 6], and southern Europe[5]. A major outbreak in Edinburgh in 1983 associated with extensive needle-sharing [5] led to 50% of PWID becoming infected [3]. This epidemic was closely linked to similar ones in Dundee and Dublin [3], but few HIV cases were seen among PWID in Glasgow at the time[7]. Rapid introduction in the UK of needle exchange in 1986 followed by other harm reduction measures [8], dramatically decreased spread of HIV within this population. Since the mid-1990s HIV diagnoses among PWID in Glasgow have averaged 10 per year [9]. Similarly in the rest of Western Europe, incidence had declined in accordance with public health responses [10].

However, in 2011 there were reports of outbreaks of HIV among PWID in Greece [11], Romania [12], and Ireland [13]. Prior to this, HIV incidence among PWID in Greece and Ireland had been similar to the UK, around 0.1% [2, 14, 15]. In Greece, fewer than 20 infections per year were reported among PWID between 2001 and 2010, but in 2011 this surged to 256 cases accounting for ¼ of all new HIV diagnoses that year [16]. The epidemics in Greece [11] and Ireland [13] followed an economic crisis which led to increases in homelessness. The recession of 2008 resulted in funding cuts to opiate substitution therapy and needle exchange programs in Greece and Romania [17]. In parallel, the surge in injection of stimulant-based new psychoactive substances, which are typically injected more frequently than heroin thus increasing the risk of needle-sharing, contributed to the outbreaks in Romania [12] and Ireland [13].

In 2015 a significant rise in HIV diagnoses among PWID was noted in Glasgow. Data from Scotland's Needle Exchange Surveillance Initiative showed that HIV prevalence among PWID increased from 0.3% in 2011-12 to 1.9% in 2015-16 [18]. Routine sequencing to test for drug resistance revealed many were HIV subtype C, a subtype rarely observed among PWID in the UK [19, 20], suggesting a common source for the outbreak.

Reconstruction of the transmission network through contact tracing is difficult for HIV because of the time delay between infection and diagnosis, the low transmission rate, and the challenge of collecting information pertaining to sexual and drug-taking behaviours. Phylogenetic analysis of viral sequences provides an alternative and independent route to reconstructing transmission networks [21]. As viral sequences are generated as a component of routine clinical care in the UK, we conducted a phylogenetic analysis to investigate whether PWID cases were related, when infections had been acquired, and whether the strain was spreading into the wider community and elsewhere in the UK. Results informed the shape and intensity of the public health response.

3 METHODS

3.1 STUDY POPULATION

Since 2005, the West of Scotland specialist virology centre has routinely carried out baseline sequencing of all new HIV diagnoses. The HIV-1 protease and reverse transcriptase regions (HXB2 positions 2253 to 3549) were amplified using primers described previously [22] with Expand reverse transcriptase and the Expand High Fidelity polymerase chain reaction (PCR) System (Roche) and the following programme: RT-PCR 42°C for 45 min; first round PCR (2 min at 94°C; 10 cycles of 15 sec at 94°C; 30 sec at 55 °C;1 min 30 sec at 72°C; 25 cycles of 15 sec at 94°C; 30 sec at 55°C; 1 min 30 sec at 72°C + 5 sec/cycle; 10 min at 72°C) and nested

PCR (2 min at 94^{o}C; 10 cycles of 15 sec at 94^{o}C; 30 sec at 55 ^{o}C;1 min 30 sec at 72^{o}C; 25 cycles of 15 sec at 94^{o}C; 30 sec at 55^{o}C; 1 min 30 sec at 72^{o}C + 5 sec/cycle; 10 min at 72^{o}C). Sanger sequencing was performed on the ABI3130xl using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Alignment and base-calling was performed using the online sequence analysis software RECall [23]. REGAv3 was used to subtype sequences and detect drug resistance mutations [24]. All subtype C sequences were extracted from the laboratory database for further analysis. At each stage (extraction through to PCR) and for each patient, negative controls were included in each assay to detect contamination. If evidence for contamination was observed, all patient samples in that run were re-extracted. For each weekly run a simple phylogenetic tree was constructed to detect contamination occurring at the sequencing stage. Any cases of sequence identity in the same batch were repeated from the extraction stage.

Avidity testing was used to classify infections as recent or older than four months. The assay is a modification of the Genscreen HIV1/2 Version 2 (Bio-Rad) [25] and testing has been performed on HIV diagnoses since 2012. Clinical and epidemiological information was obtained through the National Health Service clinical portal, a virtual electronic patient record that contains clinical notes on interactions with tertiary services and test results. Data retrieved included hepatitis C status, viral load, date of last HIV negative test, sex, risk group, age, nationality, and history of drug use, incarceration and homelessness.

3.2 BACKGROUND SEQUENCES

The UK HIV Drug Resistance Database (UKRDB) receives *pol* sequences obtained for routine clinical surveillance and submitted by participating laboratories. Resistance data are linked to demographic and clinical patient data held in the UK Collaborative HIV Cohort study (UK CHIC) database [26] and the national HIV/AIDS Reporting System (HARS) database held at Public Health England[27]. After linking sequences to epidemiological data, the data were anonymised. In the 2014 release of the database (sequences up to mid-2013), sequences were available for around 60% of the infected population and >80% of patients diagnosed since 2005. Of 63,163 sequences in the UKRDB, 15,864 sequences (25.1%) were classified as subtype C by REGAv3 [24]. Epidemiological data contributed by Public Health England and Health Protection Scotland included year of birth, gender and self-reported most likely route of infection (PWID, heterosexual sex, men who have sex with men (MSM), mother to child, blood product, or unknown).

The Los Alamos National Laboratory (LANL) HIV database compiles all published HIV sequences, including 11,658 non-UK subtype C *pol* sequences (accessed 8thAugust 2014). We used Geneious to BLAST Scottish sequences against UKRDB and LANL sequences, selecting the ten closest matches for each individual Scottish sequence for analysis [28]. As the same sequence is hit multiple times, this procedure limits the size of the final alignments. Final alignments comprised 228 sequences from Scotland, 1820 from the UKRDB and 524 from LANL (2572 in total).

3.3 GENETIC LINKAGE AND PHYLODYNAMIC ANALYSIS

Sequences were stripped of 44 sites associated with drug resistance based on the 2013 International AIDS Society list [29]. We reconstructed genetic clusters by calculating genetic distances between pairs of sequences under a TN93 substitution model. Each sequence was represented in the network by a node and nodes were linked if their pairwise distance was below a certain genetic distance threshold. At thresholds 1-2.5%, the same PWID outbreak cluster stood out (n=104, see Results), with all sequences from Scotland. We selected the tightest threshold because 1% is consistent with recent and rapid transmission [30]. 10% of outbreak sequences were submitted to Genbank (Accession numbers MG76186:MG761826). Sequences from the outbreak were further analysed using the Bayesian birth-death skyline model in BEAST2 [31, 32]. The birth-death skyline is well suited to analysing outbreaks, because unlike coalescent models, it does not assume low sample fraction within an infinite population size; instead, sample fraction is explicitly parameterized. Furthermore, the birth-death skyline estimates the effective reproductive number Re, the average number of infections originating from each individual, directly yielding epidemiologically-relevant results. Substitution models (TN93, GTR+G+I) and clock models (strict, uncorrelated lognormal) were compared and a GTR+G+I model with an uncorrelated lognormal clock was selected based on its Bayes factor. Chain samples were run for 500,000,000 generations in triplicate. Prior distributions for Re and the rate of becoming non-infectious were extracted from a previous analysis of the UK epidemic[31], and priors for the origin of the tree and the sampling proportion were based on our knowledge of the UK epidemic. The origin of the tree was set with a maximum value of 30 years and the sampling proportion was set as 0 until 2005 (the date of the first outbreak sequence) then with a normal distribution with mean=0.65 and sd=0.05. Because sampling fraction, Re and time to becoming non-infectious are correlated in the birth-death skyline model, at least one must be set with a narrow prior [31].

4 RESULTS

4.1 THE DRUG-RESISTANT SUBTYPE C OUTBREAK HAS NOT BEEN OBSERVED OUTSIDE SCOTLAND

All Scottish subtype C sequences were included in the phylogenetic analysis (n=228), alongside 1820 sequences from the UKRDB and 524 from LANL (2572 in total). Of 2572 sequences, 501 (19.5%) linked to at least one other in the network using a genetic distance cut-off of 1%.

Within the network, a tight cluster of 104 sequences stood out (Figure 1). All sequences within the cluster were less than 1% genetic distance from at least one other sequence in the cluster. Mean genetic distance was <1% with 7 patients with *pol* sequences exactly identical to another, 2 pairs and 1 triad. All sequences originated from Scotland and contained E138A and V179E. Thus we have not yet observed evidence of spread of this strain outside Scotland. E138A is a common polymorphic accessory mutation weakly selected in patients receiving etravirine and rilpivirine that reduces susceptibility to these two antiretrovirals by around two fold. V179E is a non-polymorphic mutation weakly selected by nevirapine and efavirenz and associated with resistance to nevirapine, efavirenz and etravarine. In the UKRDB, which includes sequences sampled in Scotland until mid-2013, E138A is found in 1648/15,864 of subtype C sequences (10.39%) and V179E is found in 50 (0.32%). Only 41 sequences in the UKRDB contained both mutations (0.25%), of which 26 were from the present outbreak. Among the remaining fifteen, twelve sequences comprising both mutations were not closely related to the outbreak and were not included in the phylogenetic analysis; and three were included in the analysis but did not link to the outbreak. Between 2014 and mid-2016, 5 non-outbreak HIV diagnoses were made among PWID in Scotland.

4.2 SPREAD OF HIV AMONG SCOTTISH PWID HAS BEEN RECENT AND RAPID

Sequences from the outbreak cluster (n=104) were selected for analysis using the birth-death skyline models in BEAST2 to estimate growth through time and to better infer the origin of the cluster. Runs converged within 5,000,000 generations with ESS values above 200.

The common ancestor to the cluster was dated as mid-2003 (2003.4; 95%HPD: 2001.8-2005.0), while the oldest outbreak sequence was from a female PWID diagnosed in 2005. Five patients were diagnosed in 2008-2009 (4.8%), 27 were diagnosed between 2010 and

2013 (26.0%) and 71 patients were diagnosed after 2014 (68.3%). All were diagnosed in Scotland and all those with a risk group reported were PWID.

The birth death skyline infers the effective reproductive number Re (the average number of transmissions for each individual). Importantly, Re has remained above 1 throughout the course of the outbreak (Figure 2), implying that the number of cases would be expected to continue to rise. Mean Re was estimated as 1.5 (95%HPD 0.1-3.9) over the course of the outbreak, rising to 1.8 (HPD 1.1-2.6) during the last 2 years. At its highest point, in 2009, Re exceeded 2. Sample fraction was estimated as 0.66 (HPD 0.46 -0.81).

The distance between internal nodes in the tree approximates the upper bound on time between transmission events [33]. Based on the time-resolved trees, the average transmission interval was estimated as 6.7 months for the outbreak as a whole (Supplementary Information). Looking at the phylogeny in more detail, it divided into three subclusters: 1a, 1b, and 2, originating in peak2 and 2010, respectively (Figure 3). Subclusters 1a and 1b had a higher density of recent transmission events, but there was no difference in transmission intervals between subclusters based on an analysis of 1,000 trees from the posterior distribution (Supplementary Information), indicating that while subclusters 1a and 1b are most active at present, the transmission dynamics within all three subclusters have been similar. The origin of subclusters 1a and 1b correlated with an increase in Re (Figure 2).

4.3 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF OUTBREAK MEMBERS

Among the 104 individuals in the outbreak diagnosed by mid-2016, 102 (98.1%) reported injecting drugs. Mean age was 38.4 (SD=6.5), 63/103 (61.2%) were men and 40 (38.8%) were women, 99/100 were white British (1 mixed race), 38/96 (39.6%) had a recorded history of incarceration and 41/92 (44.6%) reported having ever been homeless. 96/98 (98.0%) were confirmed to be HCV antibody positive, with 6 not tested, suggesting wide-spread sharing of injecting equipment. 68/96 (70.8%) had ongoing HCV infection with a positive HCV antigen or PCR result.

HIV avidity was tested on 87/104 (83.7%) patients and 49/104 has a previous negative test result. 24/87 (27.6%) had low avidity results indicating that infection had occurred within the last four months. Five additional patients had a date of last negative HIV test less than a year previous to their diagnosis. Three patients had antibody levels too low for avidity testing indicating acute seroconversion, confirmed with negative Western Blot and BiSpot results. Therefore, in total at least 32/87 (36.8%) had HIV for less than a year at diagnosis, consistent with the short terminal branch lengths in the phylogenetic tree (Figure 3).

5 DISCUSSION

This recent outbreak in Scotland is the latest in a series of rapid transmissions of HIV among PWID; in Greece [11], Romania [12], Ireland [13] and Indiana, USA [34]. Prior to these outbreaks, HIV incidence among PWID in these regions had been fairly static since the epidemics of the 1980s. From 2001 to 2014 there were 10-20 new cases per year in Scotland with 5-10 new cases around Glasgow [35]. The Scottish outbreak now comprises over one hundred linked cases.

Phylodynamic analysis demonstrated how rapidly the virus was transmitted, with average transmission intervals around 6 months, similar to MSM [33] and shorter than heterosexuals [36] in the UK. In contrast to the more commonly presented R0, which represents the number of onward transmissions in a fully susceptible population, Re is the number of secondary infections for the current frequency of susceptibles [37]. The number of secondary infections has averaged 1.5 since the outbreak originated around 2003, reaching 2 at its peak in 2009. In contrast, HIV diagnoses among PWID did not reach a peak until 2015 in Scotland remained around 20 per year between 2008 and 2010, not reaching a peak until

2015[38]. UK estimates of Re for heterosexuals and MSM are below 1, and just above 1, respectively [39]. Previous estimates of Re among PWID have ranged as high as 21.7 in Lithuania [40]. For the UK, estimates of Re do not exist for PWID, but Re was consistently above 1 for this outbreak, indicating that spread was ongoing in 2016. This number is specific to this outbreak and should not be extrapolated to reflect HIV transmission among PWID in the UK in general. The outbreak subdivided into subclusters, indicating independent concurrent transmission chains. All three transmission chains showed evidence of recent transmission events, and had equally short transmission intervals.

Genetic distance within the outbreak was extremely low, with multiple sets of identical partial *pol* sequences, a phenomenon observed in cases of rapid transmission [41]. While in part due to the short region of the virus sequenced, such low divergence demonstrates how rapidly the virus spread in this group. The potential for multiple partners during acute infection leads to low genetic diversity within PWID transmission networks. The recent outbreak among Greek PWID similarly displayed low diversity and high clustering[11], reminiscent of the spread of near identical subtype A variants throughout Eastern European and Russian PWID in the 1990s [42]. Full genome sequencing, currently being undertaken, may disentangle the sequence of transmissions within the outbreak.

The outbreak was in part detected because of two resistance mutations, E138A and V179E, repeatedly identified in subtype C viruses, which had not previously associated with PWID infection in the UK. This prompted a search through the UKRDB for the mutations and for related sequences. At present, despite the UKRDB collecting sequences from all HIV resistance tests, sequences are used for research purposes and not as a systematic surveillance tool. Genetic analysis confirmed the strain was unique to Scotland and is not transmitting elsewhere in the UK. In the UK it is rare to find large clusters from a single region [43], and this is now the largest cluster of HIV among PWID seen in the UK since the

1980s. However, we did not have samples from the rest of the UK as recent as those from Scotland. The most recent Scottish sequence was sampled in 2016, whereas the most recent UKRDB sequence was sampled in 2013. It is possible that the outbreak has spread outside Scotland but that we have not captured it.

No published PWID outbreaks have reported transmission of resistance mutations, although preliminary results from Saskatchewan, Canada, demonstrated the G190 mutation disproportionately affected Aboriginal PWID[44]. Earlier studies found a higher prevalence of resistance mutations among PWID than among those infected sexually[45]. Suboptimal treatment adherence among this group may provide an explanation for this phenomenon[46]. Another possibility is that blood to blood transmission could enable transmission of lower fitness viruses unable to establish infection through sexual transmission[47].

Despite access to injecting equipment, HIV still poses a significant risk to PWID. The identification of a unique strain facilitated its detection in Scotland during this outbreak, but real-time monitoring may help accelerate public health action. British Columbia recently deployed a real-time phylogeny response, where monthly reports were generated detailing cluster growth[48]. This analysis revealed a highly active cluster that expanded by eleven cases in three months. Members of the cluster were contacted to ensure linkage to care and partner notification and subsequently no further cases linked to those members were diagnosed. In the case of the Scottish outbreak, real-time phylogenetic monitoring could have brought the cluster to attention sooner. At present all UKRDB analyses are conducted with anonymised data, while Poon *et al* identified subjects to reach out to them. Use of non-anonymised HIV data for phylogenetic analyses is avoided in some jurisdictions because of the criminalisation of HIV transmission. An anonymised version of Poon’s system can also be imagined, in which the background of sequences for comparison is anonymous but data are available for the patient being seen at that moment [49]. If the patient's sequence were to cluster with two or more recent sequences, that patient could be selected for early initiation of treatment and pre-exposure prophylaxis could be offered to their partners. The advantage of Poon's method is that all members of the cluster can be retrospectively contacted whereas under the anonymised system, only patients diagnosed after the first few in a cluster would be identified. Overall, advances such as avidity testing and real-time phylogenetic analysis can be used to improve our understanding of outbreaks to better target public health responses.

Many PWID involved in the outbreak had experienced homelessness. Scotland's Needle Exchange Surveillance Initiative emphasised this point: almost 90% (20/23) of PWID from Glasgow who tested positive for HIV in 2015-16 had a history of homelessness, three-quarters of whom had been homeless within the last 6-months [18]. The situation in Scotland differs from that in other PWID outbreaks, however, because harm reduction services (Injecting Equipment Provision, Opiate Substitution Therapy) were available in Scotland post-recession. Indeed, Glasgow operates one of the most active Injecting Equipment Provision service in Europe, distributing over one million syringes per year[18]. In contrast, in Indiana, neither needle exchange nor HIV testing were available at the time of the outbreak[34]. Nonetheless, the association observed with homelessness suggests that harm reduction services available in Glasgow may have been difficult to access for those in precarious living situations, often with chaotic lifestyles.

This outbreak may have been due to a change in circumstances, but it may result from the unfortunate introduction of HIV into a group of connected but previously uninfected PWID, such as was the case in Sweden in 2006 [50] and in Indiana in 2015 [34]. The high prevalence of hepatitis C among PWID in this outbreak (>90%) is indicative of widespread injecting equipment sharing. In contrast, in Romania and Greece, multiple strains and networks were uncovered [11, 12]; these outbreaks resulted from the reduced availability of harm reduction services. The Scottish outbreak is being managed through education of the population at risk and service providers, improved addiction services, increasing provision of needle exchange (e.g. greater evening availability), improving accessibility of HIV testing, and outreach services to support early treatment and retention. Further research is needed to demonstrate whether homelessness, or other behavioural factors, played a role in the outbreak.

6 FIGURES

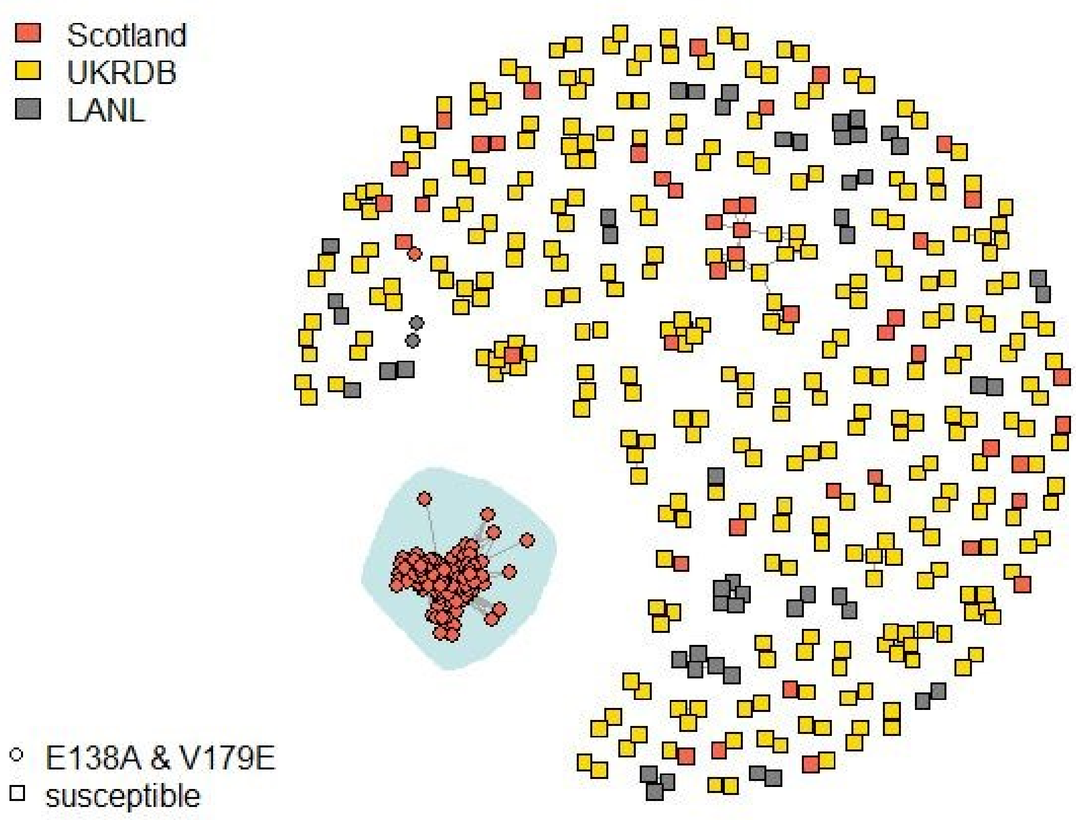

Figure 1: Genetic distance network of relatedness at 1%. Of 2572 sequences analysed, only those linked to at least one other sequence at 1% are shown (501 in total). Sequences are coloured by origin: Scotland, the UK HIV Drug Resistance Database (UKRDB) or Los Alamos National Database (LANL). Node shapes are determined by drug susceptibility of viruses.

One large cluster, highlighted and circled, stood out due to its size (104 sequences), its concentration of drug-resistant sequences and its Scottish origin.

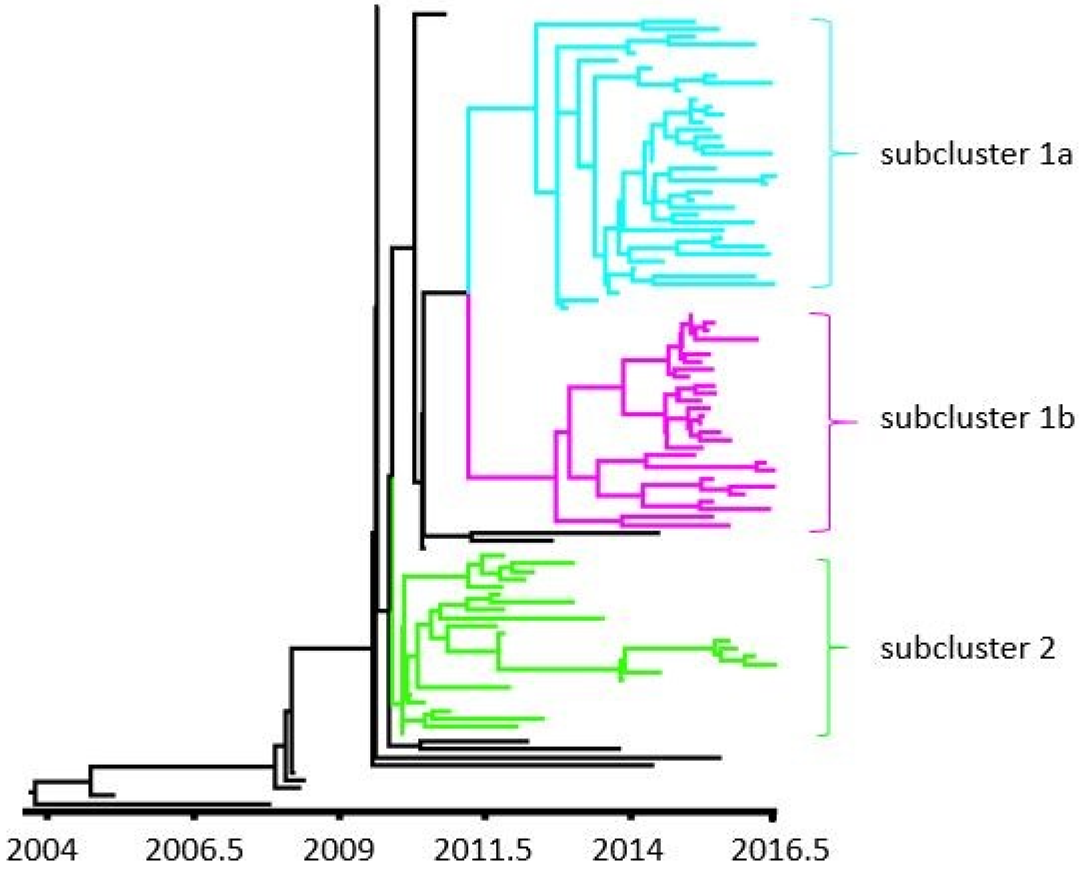

Figure 2. Reproductive number Re inferred from the birth death skyline plot. The line across the plot marks Re=1, the threshold above which an infection will continue to spread.

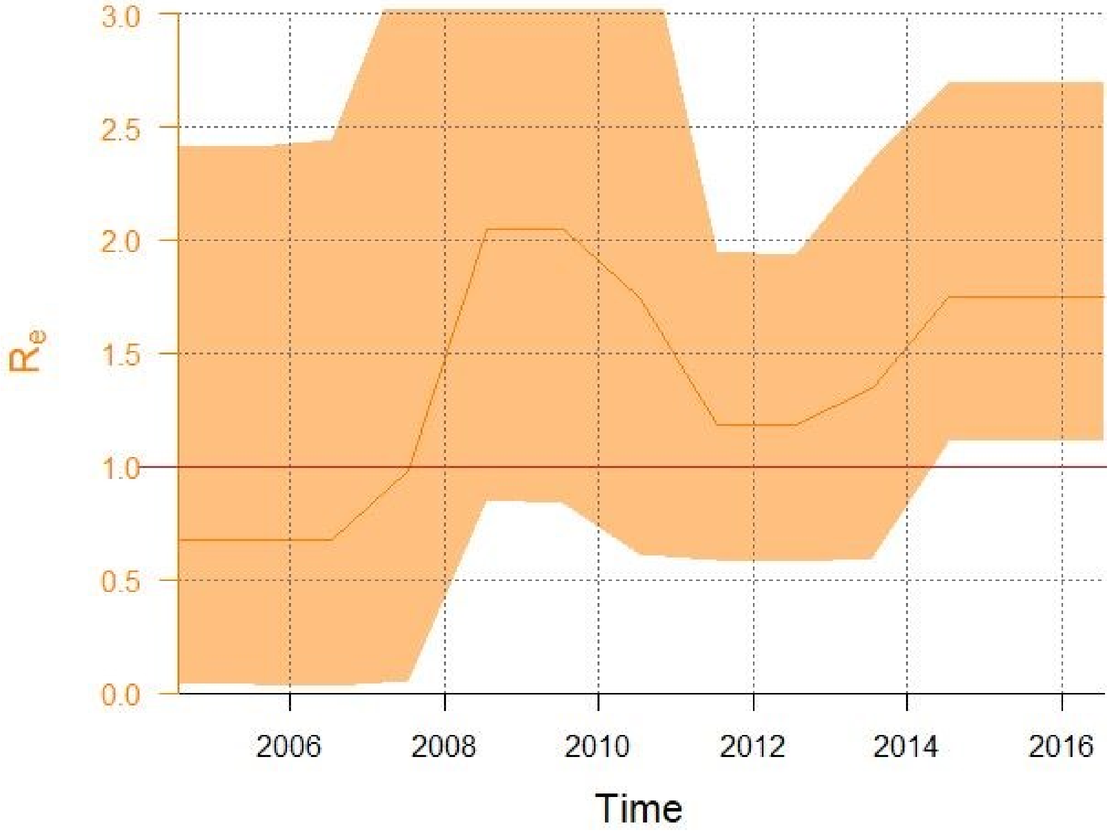

Figure 3: Time-resolved phylogeny for the outbreak cluster comprising 104 sequences from Scotland with drug resistant mutations E138A and V179E. The outbreak subdivided into three subclusters.

7 FOOTNOTES

7.1 CONFLICT OF INTEREST

MRC is currently supported by a grant from Gilead to the University of California, San Diego for work on Hepatitis C. This funding was received after the present work was completed and did not have any influence on this work. All other authors report no conflicts of interest.

7.2 MEETINGS WHERE THE WORKS HAS BEEN PRESENTED

Conference on Retroviruses and Opportunistic Infections, Seattle, USA, 2017

HIV Dynamics and Evolution, Isle of Skye, Scotland, 2017

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