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

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].

67 In 2015 a significant rise in HIV diagnoses among PWID was noted in Glasgow. Data from

68 Scotland's Needle Exchange Surveillance Initiative showed that HIV prevalence among PWID

69 increased from 0.3% in 2011-12 to 1.9% in 2015-16 [18]. Routine sequencing to test for drug

70 resistance revealed many were HIV subtype C, a subtype rarely observed among PWID in the

71 UK [19, 20], suggesting a common source for the outbreak.

72 Reconstruction of the transmission network through contact tracing is difficult for HIV

73 because of the time delay between infection and diagnosis, the low transmission rate, and

74 the challenge of collecting information pertaining to sexual and drug-taking behaviours.

75 Phylogenetic analysis of viral sequences provides an alternative and independent route to

76 reconstructing transmission networks [21]. As viral sequences are generated as a

77 component of routine clinical care in the UK, we conducted a phylogenetic analysis to

78 investigate whether PWID cases were related, when infections had been acquired, and

79 whether the strain was spreading into the wider community and elsewhere in the UK.

80 Results informed the shape and intensity of the public health response.

81 3 METHODS

82 3.1 STUDY POPULATION

83 Since 2005, the West of Scotland specialist virology centre has routinely carried out baseline

84 sequencing of all new HIV diagnoses. The HIV-1 protease and reverse transcriptase regions

85 (HXB2 positions 2253 to 3549) were amplified using primers described previously [22] with

86 Expand reverse transcriptase and the Expand High Fidelity polymerase chain reaction (PCR)

87 System (Roche) and the following programme: RT-PCR 42°C for 45 min; first round PCR (2

88 min at 94°C; 10 cycles of 15 sec at 94°C; 30 sec at 55 ^{o}C;1 min 30 sec at 72°C; 25 cycles of 15

89 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

90 PCR (2 min at 94°C; 10 cycles of 15 sec at 94°C; 30 sec at 55 ^{o}C;1 min 30 sec at 72°C; 25 cycles

91 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). Sanger

92 sequencing was performed on the ABI3130xl using BigDye Terminator v3.1 Cycle Sequencing

93 Kit (Applied Biosystems). Alignment and base-calling was performed using the online

94 sequence analysis software RECall [23]. REGAv3 was used to subtype sequences and detect

95 drug resistance mutations [24]. All subtype C sequences were extracted from the laboratory

96 database for further analysis. At each stage (extraction through to PCR) and for each patient,

97 negative controls were included in each assay to detect contamination. If evidence for

98 contamination was observed, all patient samples in that run were re-extracted. For each

99 weekly run a simple phylogenetic tree was constructed to detect contamination occurring at

100 the sequencing stage. Any cases of sequence identity in the same batch were repeated from

101 the extraction stage.

102 Avidity testing was used to classify infections as recent or older than four months. The assay

103 is a modification of the Genscreen HIV1/2 Version 2 (Bio-Rad) [25] and testing has been

104 performed on HIV diagnoses since 2012. Clinical and epidemiological information was

105 obtained through the National Health Service clinical portal, a virtual electronic patient

106 record that contains clinical notes on interactions with tertiary services and test results. Data

107 retrieved included hepatitis C status, viral load, date of last HIV negative test, sex, risk group,

108 age, nationality, and history of drug use, incarceration and homelessness.

3.2 BACKGROUND SEQUENCES

110 The UK HIV Drug Resistance Database (UKRDB) receives *pol* sequences obtained for routine

111 clinical surveillance and submitted by participating laboratories. Resistance data are linked

112 to demographic and clinical patient data held in the UK Collaborative HIV Cohort study (UK

113 CHIC) database [26] and the national HIV/AIDS Reporting System (HARS) database held at

114 Public Health England[27]. After linking sequences to epidemiological data, the data were

115   anonymised. In the 2014 release of the database (sequences up to mid-2013), sequences

116   were available for around 60% of the infected population and >80% of patients diagnosed

117   since 2005. Of 63,163 sequences in the UKRDB, 15,864 sequences (25.1%) were classified as

118   subtype C by REGAv3 [24]. Epidemiological data contributed by Public Health England and

119   Health Protection Scotland included year of birth, gender and self-reported most likely route

120   of infection (PWID, heterosexual sex, men who have sex with men (MSM), mother to child,

121   blood product, or unknown).

122   The Los Alamos National Laboratory (LANL) HIV database compiles all published HIV

123   sequences, including 11,658 non-UK subtype C *pol* sequences (accessed 8[th]August 2014). We

124   used Geneious to BLAST Scottish sequences against UKRDB and LANL sequences, selecting

125   the ten closest matches for each individual Scottish sequence for analysis [28]. As the same

126   sequence is hit multiple times, this procedure limits the size of the final alignments. Final

127   alignments comprised 228 sequences from Scotland, 1820 from the UKRDB and 524 from

128   LANL (2572 in total).

129   ## 3.3 GENETIC LINKAGE AND PHYLODYNAMIC ANALYSIS

130   Sequences were stripped of 44 sites associated with drug resistance based on the 2013

131   International AIDS Society list [29]. We reconstructed genetic clusters by calculating genetic

132   distances between pairs of sequences under a TN93 substitution model. Each sequence was

133   represented in the network by a node and nodes were linked if their pairwise distance was

134   below a certain genetic distance threshold. At thresholds 1-2.5%, the same PWID outbreak

135   cluster stood out (n=104, see Results), with all sequences from Scotland. We selected the

136   tightest threshold because 1% is consistent with recent and rapid transmission [30]. 10% of

137   outbreak sequences were submitted to Genbank (Accession numbers

138   MG76186:MG761826). Sequences from the outbreak were further analysed using the

139   Bayesian birth-death skyline model in BEAST2 [31, 32]. The birth-death skyline is well suited

140   to analysing outbreaks, because unlike coalescent models, it does not assume low sample

141   fraction within an infinite population size; instead, sample fraction is explicitly

142   parameterized. Furthermore, the birth-death skyline estimates the effective reproductive

143   number Re, the average number of infections originating from each individual, directly

144   yielding epidemiologically-relevant results. Substitution models (TN93, GTR+G+I) and clock

145   models (strict, uncorrelated lognormal) were compared and a GTR+G+I model with an

146   uncorrelated lognormal clock was selected based on its Bayes factor. Chain samples were

147   run for 500,000,000 generations in triplicate. Prior distributions for Re and the rate of

148   becoming non-infectious were extracted from a previous analysis of the UK epidemic[31],

149   and priors for the origin of the tree and the sampling proportion were based on our

150   knowledge of the UK epidemic. The origin of the tree was set with a maximum value of 30

151   years and the sampling proportion was set as 0 until 2005 (the date of the first outbreak

152   sequence) then with a normal distribution with mean=0.65 and sd=0.05. Because sampling

153   fraction, Re and time to becoming non-infectious are correlated in the birth-death skyline

154   model, at least one must be set with a narrow prior [31].

155   # 4 RESULTS

156   ## 4.1 THE DRUG-RESISTANT SUBTYPE C OUTBREAK HAS NOT BEEN OBSERVED

157   ## OUTSIDE SCOTLAND

158   All Scottish subtype C sequences were included in the phylogenetic analysis (n=228),

159   alongside 1820 sequences from the UKRDB and 524 from LANL (2572 in total). Of 2572

160   sequences, 501 (19.5%) linked to at least one other in the network using a genetic distance

161   cut-off of 1%.

162   Within the network, a tight cluster of 104 sequences stood out (Figure 1). All sequences

163   within the cluster were less than 1% genetic distance from at least one other sequence in

164   the cluster. Mean genetic distance was <1% with 7 patients with *pol* sequences exactly

165   identical to another, 2 pairs and 1 triad. All sequences originated from Scotland and

166   contained E138A and V179E. Thus we have not yet observed evidence of spread of this

167   strain outside Scotland. E138A is a common polymorphic accessory mutation weakly

168   selected in patients receiving etravirine and rilpivirine that reduces susceptibility to these

169   two antiretrovirals by around two fold. V179E is a non-polymorphic mutation weakly

170   selected by nevirapine and efavirenz and associated with resistance to nevirapine, efavirenz

171   and etravarine. In the UKRDB, which includes sequences sampled in Scotland until mid-2013,

172   E138A is found in 1648/15,864 of subtype C sequences (10.39%) and V179E is found in 50

173   (0.32%). Only 41 sequences in the UKRDB contained both mutations (0.25%), of which 26

174   were from the present outbreak. Among the remaining fifteen, twelve sequences comprising

175   both mutations were not closely related to the outbreak and were not included in the

176   phylogenetic analysis; and three were included in the analysis but did not link to the

177   outbreak. Between 2014 and mid-2016, 5 non-outbreak HIV diagnoses were made among

178   PWID in Scotland.

179   **4.2 SPREAD OF HIV AMONG SCOTTISH PWID HAS BEEN RECENT AND RAPID**

180   Sequences from the outbreak cluster (n=104) were selected for analysis using the birth-

181   death skyline models in BEAST2 to estimate growth through time and to better infer the

182   origin of the cluster. Runs converged within 5,000,000 generations with ESS values above

183   200.

184   The common ancestor to the cluster was dated as mid-2003 (2003.4; 95%HPD: 2001.8-

185   2005.0), while the oldest outbreak sequence was from a female PWID diagnosed in 2005.

186   Five patients were diagnosed in 2008-2009 (4.8%), 27 were diagnosed between 2010 and

187 2013 (26.0%) and 71 patients were diagnosed after 2014 (68.3%). All were diagnosed in

188 Scotland and all those with a risk group reported were PWID.

189 The birth death skyline infers the effective reproductive number Re (the average number of

190 transmissions for each individual). Importantly, Re has remained above 1 throughout the

191 course of the outbreak (Figure 2), implying that the number of cases would be expected to

192 continue to rise. Mean Re was estimated as 1.5 (95%HPD 0.1-3.9) over the course of the

193 outbreak, rising to 1.8 (HPD 1.1-2.6) during the last 2 years. At its highest point, in 2009, Re

194 exceeded 2. Sample fraction was estimated as 0.66 (HPD 0.46 -0.81).

195 The distance between internal nodes in the tree approximates the upper bound on time

196 between transmission events [33]. Based on the time-resolved trees, the average

197 transmission interval was estimated as 6.7 months for the outbreak as a whole

198 (Supplementary Information). Looking at the phylogeny in more detail, it divided into three

199 subclusters: 1a, 1b, and 2, originating in peak2 and 2010, respectively (Figure 3). Subclusters

200 1a and 1b had a higher density of recent transmission events, but there was no difference in

201 transmission intervals between subclusters based on an analysis of 1,000 trees from the

202 posterior distribution (Supplementary Information), indicating that while subclusters 1a and

203 1b are most active at present, the transmission dynamics within all three subclusters have

204 been similar. The origin of subclusters 1a and 1b correlated with an increase in Re (Figure 2).

205 ## 4.3 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF OUTBREAK MEMBERS

206 Among the 104 individuals in the outbreak diagnosed by mid-2016, 102 (98.1%) reported

207 injecting drugs. Mean age was 38.4 (SD=6.5), 63/103 (61.2%) were men and 40 (38.8%) were

208 women, 99/100 were white British (1 mixed race), 38/96 (39.6%) had a recorded history of

209 incarceration and 41/92 (44.6%) reported having ever been homeless. 96/98 (98.0%) were

210 confirmed to be HCV antibody positive, with 6 not tested, suggesting wide-spread sharing of

211   injecting equipment. 68/96 (70.8%) had ongoing HCV infection with a positive HCV antigen

212   or PCR result.

213   HIV avidity was tested on 87/104 (83.7%) patients and 49/104 has a previous negative test

214   result. 24/87 (27.6%) had low avidity results indicating that infection had occurred within

215   the last four months. Five additional patients had a date of last negative HIV test less than a

216   year previous to their diagnosis. Three patients had antibody levels too low for avidity

217   testing indicating acute seroconversion, confirmed with negative Western Blot and BiSpot

218   results. Therefore, in total at least 32/87 (36.8%) had HIV for less than a year at diagnosis,

219   consistent with the short terminal branch lengths in the phylogenetic tree (Figure 3).

5 DISCUSSION

221   This recent outbreak in Scotland is the latest in a series of rapid transmissions of HIV among

222   PWID; in Greece [11], Romania [12], Ireland [13] and Indiana, USA [34]. Prior to these

223   outbreaks, HIV incidence among PWID in these regions had been fairly static since the

224   epidemics of the 1980s. From 2001 to 2014 there were 10-20 new cases per year in Scotland

225   with 5-10 new cases around Glasgow [35]. The Scottish outbreak now comprises over one

226   hundred linked cases.

227   Phylodynamic analysis demonstrated how rapidly the virus was transmitted, with average

228   transmission intervals around 6 months, similar to MSM [33] and shorter than heterosexuals

229   [36] in the UK. In contrast to the more commonly presented R0, which represents the

230   number of onward transmissions in a fully susceptible population, Re is the number of

231   secondary infections for the current frequency of susceptibles [37]. The number of

232   secondary infections has averaged 1.5 since the outbreak originated around 2003, reaching

233   2 at its peak in 2009. In contrast, HIV diagnoses among PWID did not reach a peak until 2015

234   in Scotland remained around 20 per year between 2008 and 2010, not reaching a peak until

235 2015[38]. UK estimates of Re for heterosexuals and MSM are below 1, and just above 1,

236 respectively [39]. Previous estimates of Re among PWID have ranged as high as 21.7 in

237 Lithuania [40]. For the UK, estimates of Re do not exist for PWID, but Re was consistently

238 above 1 for this outbreak, indicating that spread was ongoing in 2016. This number is

239 specific to this outbreak and should not be extrapolated to reflect HIV transmission among

240 PWID in the UK in general. The outbreak subdivided into subclusters, indicating independent

241 concurrent transmission chains. All three transmission chains showed evidence of recent

242 transmission events, and had equally short transmission intervals.

243 Genetic distance within the outbreak was extremely low, with multiple sets of identical

244 partial *pol* sequences, a phenomenon observed in cases of rapid transmission [41]. While in

245 part due to the short region of the virus sequenced, such low divergence demonstrates how

246 rapidly the virus spread in this group. The potential for multiple partners during acute

247 infection leads to low genetic diversity within PWID transmission networks. The recent

248 outbreak among Greek PWID similarly displayed low diversity and high clustering[11],

249 reminiscent of the spread of near identical subtype A variants throughout Eastern European

250 and Russian PWID in the 1990s [42]. Full genome sequencing, currently being undertaken,

251 may disentangle the sequence of transmissions within the outbreak.

252 The outbreak was in part detected because of two resistance mutations, E138A and V179E,

253 repeatedly identified in subtype C viruses, which had not previously associated with PWID

254 infection in the UK. This prompted a search through the UKRDB for the mutations and for

255 related sequences. At present, despite the UKRDB collecting sequences from all HIV

256 resistance tests, sequences are used for research purposes and not as a systematic

257 surveillance tool. Genetic analysis confirmed the strain was unique to Scotland and is not

258 transmitting elsewhere in the UK. In the UK it is rare to find large clusters from a single

259 region [43], and this is now the largest cluster of HIV among PWID seen in the UK since the

260   1980s. However, we did not have samples from the rest of the UK as recent as those from

261   Scotland. The most recent Scottish sequence was sampled in 2016, whereas the most recent

262   UKRDB sequence was sampled in 2013. It is possible that the outbreak has spread outside

263   Scotland but that we have not captured it.

264   No published PWID outbreaks have reported transmission of resistance mutations, although

265   preliminary results from Saskatchewan, Canada, demonstrated the G190 mutation

266   disproportionately affected Aboriginal PWID[44]. Earlier studies found a higher prevalence

267   of resistance mutations among PWID than among those infected sexually[45]. Suboptimal

268   treatment adherence among this group may provide an explanation for this

269   phenomenon[46]. Another possibility is that blood to blood transmission could enable

270   transmission of lower fitness viruses unable to establish infection through sexual

271   transmission[47].

272   Despite access to injecting equipment, HIV still poses a significant risk to PWID. The

273   identification of a unique strain facilitated its detection in Scotland during this outbreak, but

274   real-time monitoring may help accelerate public health action. British Columbia recently

275   deployed a real-time phylogeny response, where monthly reports were generated detailing

276   cluster growth[48]. This analysis revealed a highly active cluster that expanded by eleven

277   cases in three months. Members of the cluster were contacted to ensure linkage to care and

278   partner notification and subsequently no further cases linked to those members were

279   diagnosed. In the case of the Scottish outbreak, real-time phylogenetic monitoring could

280   have brought the cluster to attention sooner. At present all UKRDB analyses are conducted

281   with anonymised data, while Poon *et al* identified subjects to reach out to them. Use of non-

282   anonymised HIV data for phylogenetic analyses is avoided in some jurisdictions because of

283   the criminalisation of HIV transmission. An anonymised version of Poon's system can also be

284   imagined, in which the background of sequences for comparison is anonymous but data are

285 available for the patient being seen at that moment [49]. If the patient's sequence were to

286 cluster with two or more recent sequences, that patient could be selected for early initiation

287 of treatment and pre-exposure prophylaxis could be offered to their partners. The

288 advantage of Poon's method is that all members of the cluster can be retrospectively

289 contacted whereas under the anonymised system, only patients diagnosed after the first

290 few in a cluster would be identified. Overall, advances such as avidity testing and real-time

291 phylogenetic analysis can be used to improve our understanding of outbreaks to better

292 target public health responses.

293 Many PWID involved in the outbreak had experienced homelessness. Scotland's Needle

294 Exchange Surveillance Initiative emphasised this point: almost 90% (20/23) of PWID from

295 Glasgow who tested positive for HIV in 2015-16 had a history of homelessness, three-

296 quarters of whom had been homeless within the last 6-months [18]. The situation in

297 Scotland differs from that in other PWID outbreaks, however, because harm reduction

298 services (Injecting Equipment Provision, Opiate Substitution Therapy) were available in

299 Scotland post-recession. Indeed, Glasgow operates one of the most active Injecting

300 Equipment Provision service in Europe, distributing over one million syringes per year[18]. In

301 contrast, in Indiana, neither needle exchange nor HIV testing were available at the time of

302 the outbreak[34]. Nonetheless, the association observed with homelessness suggests that

303 harm reduction services available in Glasgow may have been difficult to access for those in

304 precarious living situations, often with chaotic lifestyles.

305 This outbreak may have been due to a change in circumstances, but it may result from the

306 unfortunate introduction of HIV into a group of connected but previously uninfected PWID,

307 such as was the case in Sweden in 2006 [50] and in Indiana in 2015 [34]. The high prevalence

308 of hepatitis C among PWID in this outbreak (>90%) is indicative of widespread injecting

309 equipment sharing. In contrast, in Romania and Greece, multiple strains and networks were

310 uncovered [11, 12]; these outbreaks resulted from the reduced availability of harm

311 reduction services. The Scottish outbreak is being managed through education of the

312 population at risk and service providers, improved addiction services, increasing provision of

313 needle exchange (e.g. greater evening availability), improving accessibility of HIV testing, and

314 outreach services to support early treatment and retention. Further research is needed to

315 demonstrate whether homelessness, or other behavioural factors, played a role in the
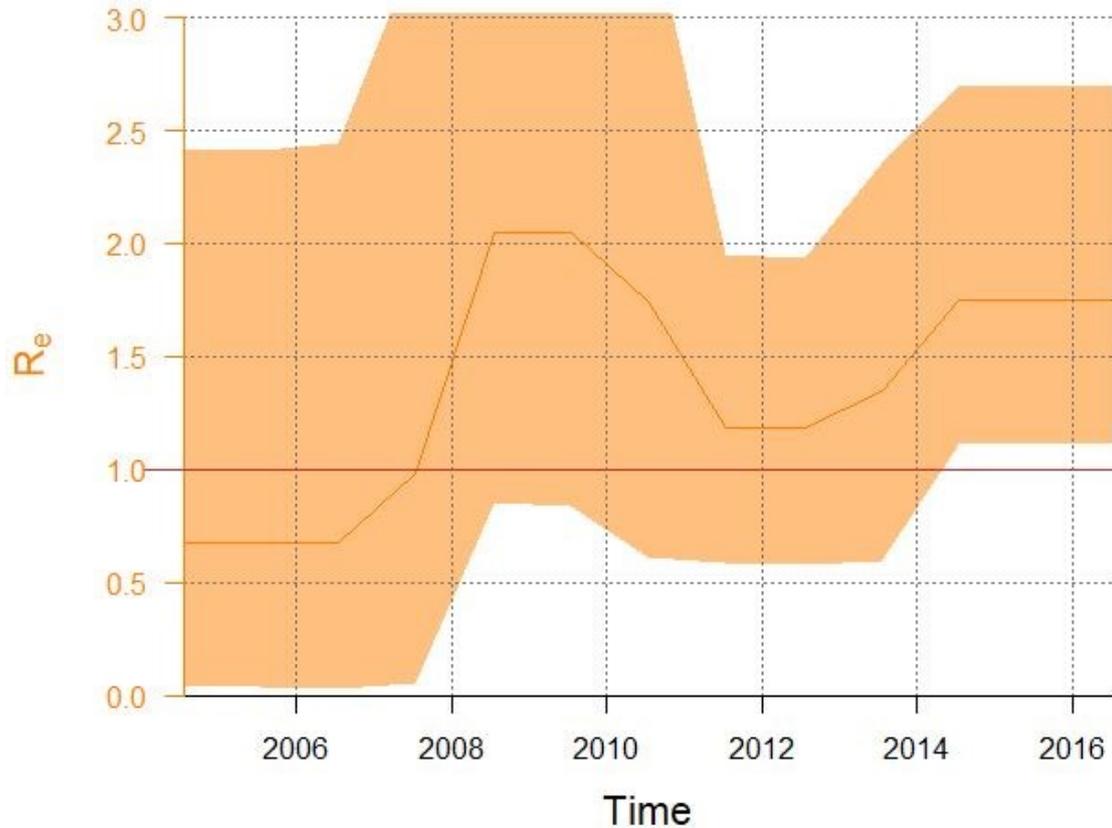
316 outbreak.
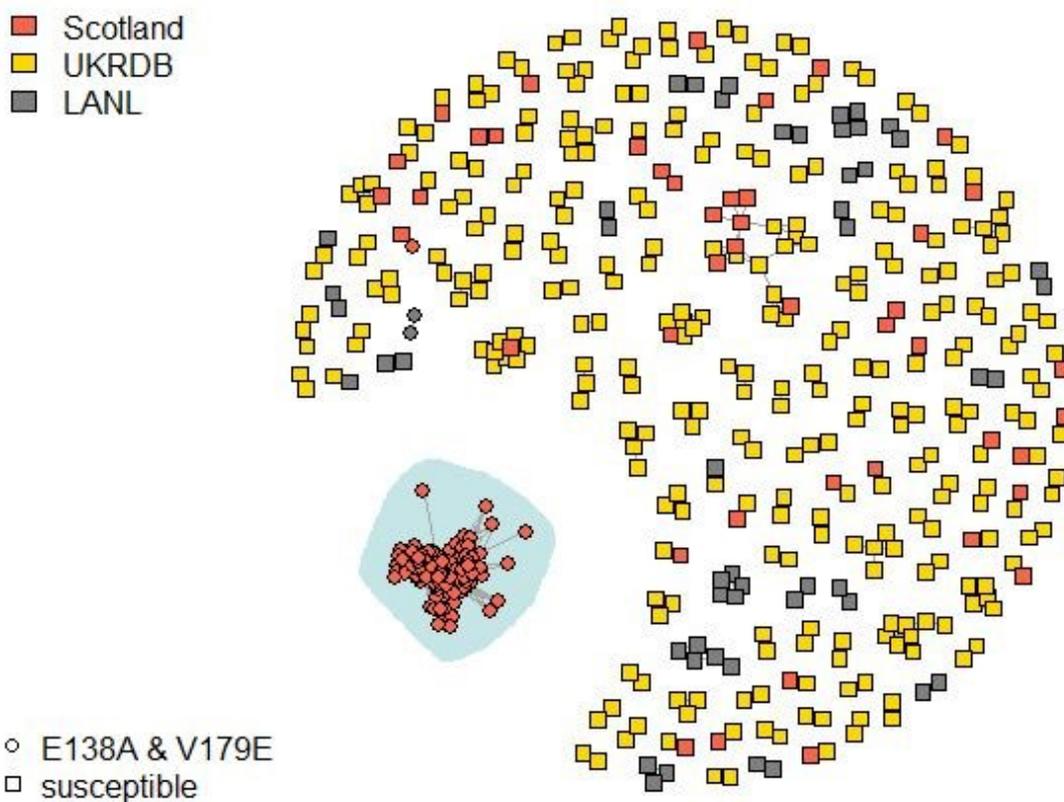
6 FIGURES

318

319 Figure 1: Genetic distance network of relatedness at 1%. Of 2572 sequences analysed, only

320 those linked to at least one other sequence at 1% are shown (501 in total). Sequences are

321 coloured by origin: Scotland, the UK HIV Drug Resistance Database (UKRDB) or Los Alamos

322 National Database (LANL). Node shapes are determined by drug susceptibility of viruses.

14

323   One large cluster, highlighted and circled, stood out due to its size (104 sequences), its

324   concentration of drug-resistant sequences and its Scottish origin.

325
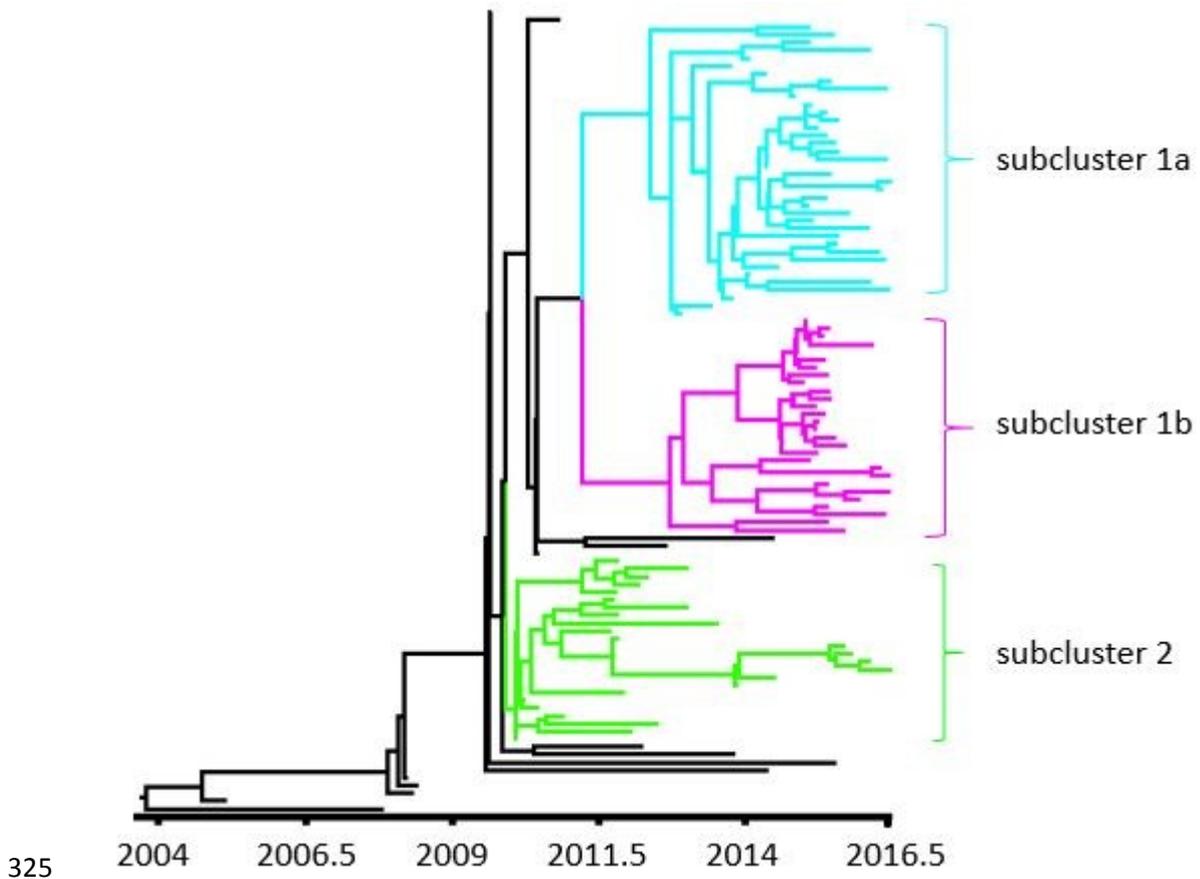
326   Figure 2. Reproductive number Re inferred from the birth death skyline plot. The line across

327   the plot marks Re=1, the threshold above which an infection will continue to spread.

328

329

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

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

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