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## **Clonal replacement and expansion among invasive meningococcal isolates of serogroup W in France**

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## 1 **Abstract**

### 2 **Background**

3 *Neisseria meningitidis* group W (NmW) belonging to the clonal complex ST-11 (NmW/cc11)  
4 spread in Europe and in France in 2000 and declined thereafter. In France, invasive  
5 meningococcal disease (IMD) due to NmW increased again in 2012 and thereafter since 2015.  
6 Several sub-lineages of NmW/cc11 are circulating worldwide with successive epidemic waves.  
7 We aimed to describe recent epidemiological trends of NmW in France and to explore the  
8 microbiological and epidemiological characteristics associated with different NmW/cc11 sub-  
9 lineages.

### 10 **Methods**

11 The epidemiology of NmW was described based on data collected through mandatory  
12 notification of IMD and strain typing data for culture-confirmed and PCR-confirmed cases for the  
13 period 2000-2016. All culture-confirmed cases due to NmW from the period 2010-2016 were  
14 characterized by whole genome sequencing (WGS). A detailed epidemiological analysis was  
15 performed for culture-confirmed cases on the basis of WGS data.

### 16 **Findings**

17 During the period 2010-2016, genotyping was obtained for 148 cases including all the 132  
18 culture-confirmed cases, among which 127 were matched with epidemiological data, and 16  
19 PCR-confirmed cases (out of a total of 47 PCR-confirmed cases). An increase in IMD was  
20 observed in 2012 and was linked to isolates belonging to the “Anglo-French-Hajj” sub-lineage.  
21 These isolates have decreased significantly since 2013 and have been replaced by NmW/cc11  
22 isolates related to the “South American – UK” sub-lineage which caused a marked increase in the  
23 number of cases of NmW in 2016. In this sub-lineage, the “original UK strain” was first detected  
24 in 2012 and increased thereafter, followed by the recently described “UK 2013-strain”. Isolates

25 related to the “South American-UK” sub-lineage represented 45% of all NmW cultured isolates  
26 from the whole period 2010-2016 but were the most frequent isolates in 2016, representing 76%  
27 of the total NmW typed isolates and 94% of the typed NmW/cc11 isolates. A changing pattern in  
28 the epidemiology of NmW has been observed in 2015-2016 in relation to the spread of the “UK  
29 2013-strain” with a sharp increase in the number of cases among persons aged 15 years and over  
30 and a high case fatality rate (CFR) . Among cases due to the “UK 2013-strain”, 94% of cases  
31 were aged 15 years and over and the CFR was 28%.

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### 33 **Interpretation**

34 Our data suggest a recent clonal replacement among NmW/cc11 isolates with the expansion of  
35 the “South American-UK” sub-lineage in France and particularly the “UK 2013-strain” which  
36 was predominant in 2015 and 2016. A shift in the age-distribution of IMD due to NmW to older  
37 ages and the high CFR are consistent with the expansion of a new virulent clone in a naive  
38 population. These data may have an impact on tailoring vaccination strategies against NmW.

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49 **Highlights**

50 *Neisseria meningitidis* group W is currently increasing in Europe and in France.

51 Previous increases in France were linked to the Hajj and to travel to Africa.

52 Whole genome sequencing was used to characterise W isolates in France in 2010-2016.

53 The “UK 2013-strain” has recently expanded in adults aged 15 years and over.

54 A high case fatality rate was observed among cases caused by the “UK 2013-strain”.

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## 73 **Introduction**

74 Invasive meningococcal disease (IMD) is caused by the Gram negative bacterium  
75 *Neisseria meningitidis* (Nm) and mainly manifests as septicaemia and meningitis. This bacterium  
76 is frequently hosted in the nasopharynx (1). Invasive isolates (responsible for IMD) are usually  
77 capsulated while carriage isolates are frequently non-capsulated (2). The polysaccharide  
78 composition of the capsule defines the serogroup. Twelve serogroups are currently described but  
79 6 of them (A, B, C, Y, W and X) are responsible for almost all IMD worldwide with  
80 high geographic variation (3, 4). Vaccines based on the capsular polysaccharides are available  
81 against serogroups A, C, Y and W meningococci while protein-based vaccines target  
82 serogroup B meningococci (5).

83 In France, routine vaccination with meningococcal C conjugate vaccines (MCCV) was  
84 introduced into the immunization schedule in early 2010 for infants at 12 months of age and a  
85 catch-up until 24-years-old (one dose of MCCV). Vaccination against meningococci ACWY and  
86 against meningococci B are recommended for at risk subjects and outbreaks control (6).

87 The management of IMD requires both treatment of patients and prophylactic measures  
88 among close contacts to avoid secondary spread. Surveillance is also a key element to  
89 better tailor vaccination strategies. Indeed, IMD occurs as sporadic cases with occasional  
90 outbreaks in Europe and North America while major epidemics periodically occur in Sub-  
91 Saharan Africa (7). Meningococcal isolates showed high diversity with occasional global  
92 spread of epidemic strains belonging to different serogroups (7). Serogroup W (NmW) isolates  
93 have been detected since the late 70s but historically caused a minor proportion of IMD  
94 cases globally. In 2000, NmW underwent a first global spread among Hajj pilgrims and their  
95 contacts (8). Subsequently NmW spread in the African Sub-Saharan meningitis belt and  
caused major outbreaks in Sub-Saharan

96 countries at the beginning of the 2000s and in 2010 (9-11). During the 2000 decade, NmW  
97 also emerged in the South America Cone and spread to the United Kingdom (UK) in 2009  
98 (12-14). Tracking these isolates requires powerful typing methods such as multilocus  
99 sequence typing (MLST) based originally on the sequence of 7 meningococcal genes. MLST  
100 determines sequence types (ST) that can be clustered into clonal complexes (cc) (15). Several  
101 clonal complexes have been identified but most NmW outbreak isolates belong to the  
102 clonal complex cc11 (cc22 maintains a steady presence in many countries) (7, 12). However,  
103 MLST may lack resolution to discriminate clones that can be resolved by whole genome  
104 sequencing (WGS) (16). Core genome MLST (cgMLST) has already been used to describe the  
105 the global spread of W/cc11 lineage in several countries over decades and allowed the  
106 characterisation of distinct sub-lineages within W/cc11 isolates. In particular, it showed the  
107 emergence of the “South American – UK” sub-lineage in UK which has been further  
108 characterised in two distinct strains: the “original UK strain” which emerged in UK in 2009  
109 and the “UK 2013-strain” which emerged in UK in 2013 (17).

110 In France, studying the characteristics of NmW/cc11 is particularly interesting as various W/cc11  
111 sub-lineages have been co-circulating in the country in addition to NmW isolates belonging to  
112 other clonal complexes (10, 18, 19). A thorough genetic analysis of NmW isolates combined with  
113 epidemiological data on cases may unravel differential spread/virulence/replacement abilities.  
114 We aimed to explore the characteristics of NmW IMD cases caused by these different lineages  
115 and sub-lineages in France since 2010.

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## 120 **Methods**

### 121 **Surveillance of IMD in France**

122 The French surveillance system of IMD is based on the mandatory notification of IMD cases on  
123 the basis of a standardized case definition. Cases are notified to the Regional Health Agencies  
124 which implement local control measures. The notification forms are then sent to Santé publique  
125 France, the French national public health agency, for data entry and analysis for the purpose of  
126 epidemiological surveillance. IMD cases are notified on the basis of at least one of the following  
127 criteria: isolation of *N. meningitidis* or detection of *N. meningitidis* nucleic acid by PCR (PCR  
128 was introduced in 2002 in the case definition) from a normally sterile site (blood, cerebrospinal  
129 fluid (CSF), other sterile sites or purpuric skin lesions) ; detection of Gram-negative stained  
130 diplococci in CSF; purulent CSF associated with purpuric skin lesions or with the detection of  
131 *N. meningitidis* antigens in blood, urine or CSF (antigen detection was not used anymore  
132 after 2014); *purpura fulminans* (severe sepsis with extensive hemorrhagic and necrotic skin  
133 lesions) (19). In addition, *N. meningitidis* isolates, as well as PCR positive samples are  
134 sent to the National Reference Centre for Meningococci (NRCM) for confirmation and full  
135 strain typing. Meningococcal groups are determined by agglutination of cultured isolates  
136 using specific “in house” rabbit sera and by PCR for non-cultured confirmed cases (20).  
137 Throughout the manuscript “group” will be used to refer to both serogroup and genogroup to  
138 characterise cultured-confirmed and PCR-confirmed cases respectively. PCR confirmation and  
139 typing techniques performed at the NRCM include PCR targeting *N. meningitidis*-specific  
140 genes as well as capsular genes for serogroup determination (*ctrA*, *sodC*, *csaB*, *csB*, *csC*,  
141 *csW*, *csY* and *csxA*).

142

### **Genomic analysis**

143 The NRCM performs systematically MLST analysis on all IMD cases (cultured and PCR-  
144 confirmed cases) since 2010 as previously described (18, 20, 21). Typing data are expressed as a  
145 genetic formula: G :P1.PorA-VR1, PorA-VR2:FetA :cc that define the group (G), the two  
146 variable regions (VR1 and VR2) of the outer membrane protein PorA, and of one VR of the  
147 protein FetA, as well as the cc.

148 Moreover, WGS is also performed systematically on all invasive cultured isolates since late 2015.  
149 For this work we retrospectively performed WGS on all cultured NmW isolates since 2010.  
150 Genomic DNA was extracted by using a Genomic-tip 20/G kit (QIAGEN, Valencia CA, USA).  
151 Sequencing was performed by Illumina HiSeq 2000 sequencer (Illumina, San Diego, CA, USA)  
152 and assembled as previously described (21). Sequences are available through the PubMLST  
153 database which runs on the Bacterial Isolate Genome Sequence Database (BIGSdb) platform  
154 (22). WGS data was analyzed using a “gene-by-gene” approach available through the PubMLST  
155 Genome Comparator tool using *N. meningitidis* core genome v1.0 that includes 1605 core loci  
156 (22). SplitsTree4 (version 4.13.1) was used to visualise the resulting distance matrices as  
157 Neighbour-net networks (23). For the genomic analysis we included three reference strains  
158 according to Lucidarme et al (24): one French isolate related to the “Anglo-French Hajj” sub-  
159 lineage (ID31165), one isolate related to the “original UK strain” (ID20196) and one isolate  
160 related to the “UK 2013-strain” (ID30167). The IDs of all these isolates are given in the  
161 supplementary Table to allow retrieving of WGS sequence in FASTA formats.

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### 163 **Study period**

164 Two periods were considered: (i) 2000-2016 for the description of the epidemiological trends of  
165 NmW IMD and MLST and (ii) 2010-2016 for the whole genome analysis and the characteristics  
166 of cases according to the different sub-lineages.

167 **Data analysis**

168 Two databases were used in this study. The first one relates to IMD cases reported through  
169 mandatory notification and includes clinical, epidemiological data as well as microbiological data  
170 (technique for diagnosis and group identified by the local hospital or by the NRCM). The second  
171 database includes data on all NmW invasive strains characterized by the NRCM. These two  
172 databases are highly dependent but do not completely overlap. Cases in the database of the  
173 NRCM included PCR since 1998 while PCR was added on the criteria of the mandatory  
174 reporting in 2002. Data analysis was performed distinctly using these two databases except for  
175 the analysis of characteristics of cases by lineages/sub-lineages for which the databases were  
176 merged.

177 Epidemiological and clinical characteristics of cases were studied by groups (NmW versus other  
178 groups) and by NmW lineages and sub-lineages identified by WGS. Proportions were compared  
179 using Chi-square test or Fisher exact test using the threshold of p-value  $< 0.05$  as statistically  
180 significant. Poisson regression was used to estimate the relative risk (RR) for death by group after  
181 adjusting for age. Statistical analysis was performed using Stata version 12.1 software.

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## 191 **Results**

### 192 **Epidemiology of NmW IMD**

193 Between 2000 and 2016, the number of cases fluctuated in relation with the global spread of  
194 NmW and multinational outbreaks (Fig.1). In 2000-2003, a first increase was described in  
195 relation to the global Hajj-associated outbreak (8, 18). Then, from 2003 to 2011, there was a  
196 marked decreasing trend and at the lowest level in 2009-2011, NmW caused around 10-15 cases  
197 per year representing 2% of all IMD cases (Fig. 1). In 2012, there was a transient increase in the  
198 number of cases in relation to outbreaks in African Sub-Saharan countries. In 2015 and 2016, a  
199 new upsurge was observed. In 2016, 45 cases were reported (incidence rate of 0.07 / 100 000  
200 inhabitants). NmW remained the least frequent group identified but the proportion of cases due to  
201 NmW increased compared to previous years. In 2016, of 506 IMD cases with known group, 52%  
202 of IMD belonged to group B, 26% to group C, 12% to group Y and 9% to group W.

203  
204 In 2015-2016, there were significant differences in characteristics of IMD cases according to the  
205 strain group (Table 1). The proportion of cases in age groups above 15 years old was higher for  
206 group W compared to group B and C cases. NmW IMD was associated with a higher CFR  
207 (22.1% vs 10.4% for other groups,  $p=0.002$ ). After adjusting for age, the CFR was still greater  
208 among group W cases than other groups (RR for death = 1.2 [95% IC 1.01-1.42]). In comparison  
209 with group B and C cases, group W cases presented more frequently with septicemia and less  
210 frequently with meningitis (Table 1). The proportion of cases with Nm isolated from synovial  
211 fluid was greater among NmW cases.

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### 213 **Molecular characteristics of NmW invasive meningococcal isolates**

214 During the period 2000-2016 the NRCM received samples for 527 cases of NmW IMD : 480  
215 (91%) cases were cultured-confirmed and 47 (9%) were only PCR-confirmed (MLST performed  
216 on PCR-confirmed cases since 2010). The annual distribution of cases is shown in Fig. 2. MLST  
217 data were available for 366 cases (69%) including all the cultured-confirmed cases since 2010  
218 (n=132) and 16 of the 47 cases that were only PCR-confirmed.

219 Among the 366 cases with MLST data, cc11 was the most frequent and caused 195 cases (53%)  
220 followed by cc22 with 128 cases (35%) while other cc were responsible for 43 cases (12%). The  
221 cc11 isolates were responsible for the increase of NmW IMD observed at the beginning of the  
222 2000s, in 2012 and in 2016 (Fig. 1 and Fig. 2). The number of NmW/cc11 cases was particularly  
223 high in the years 2000, 2001 and 2016. Over all the study period, NmW/cc11 isolates showed the  
224 same genotypic formula (W:P1.5,2:F1-1:cc11). NmW/cc22 isolates were more heterogeneous.

225

## 226 **Genomic analysis of NmW cultured isolates**

227 To describe the evolutionary relationships of the isolates and particularly those of the cc11, all  
228 132 cultured isolates (from the period 2010-2016) were subjected to WGS and the data were  
229 uploaded to the PubMLST database and analysed using the BIGSdb tools in this database.  
230 Isolates grouped into two major clusters corresponding to cc11 and cc22. Isolates belonging to  
231 other cc were distinct from these two clusters (Fig. 3A). Isolates belonging to “cc22” and to  
232 “other cc” showed a higher diversity than “cc11” as suggested by their genetic distances (Fig.3 B  
233 and C). Eighty-four isolates belonged to cc11 (of which 70 were identical by MLST) and  
234 showed differentiation into two groups that corresponded to the “Anglo-French-Hajj” sub-  
235 lineage and to the “South American-UK” sub-lineage (Fig 3A). Few cc11 isolates were  
236 separated from the above mentioned two lineages and may correspond to other local cc11  
237 isolates that were related to the “Anglo-French-Hajj” sub-lineage (Fig. 3D dashed-line circle). It  
is noteworthy that isolates

238 belonging to the “Anglo-French-Hajj” sub-lineage were distributed throughout 2011-2016 (no  
239 cc11 was detected in 2010 among all typed cases). In particular, the numbers of isolates related to  
240 the “Anglo-French Hajj” strain increased in 2012-2013 and decreased thereafter (only two  
241 isolates in 2016) (Fig. 3D). On the other hand, the “South American-UK” isolates were first  
242 detected in 2012 and increased thereafter (Fig. 3E). The first case was detected in a patient from  
243 United Kingdom who was in holiday in France in June 2012. Isolates belonging to the “South  
244 American-UK” sub-lineage represented 45% of all NmW cultured isolates from the whole period  
245 2010-2016. They increased after 2012 and were the most frequent NmW/cc11 isolates in France  
246 in 2016, representing 34 isolates (76%) of the 45 NmW typed isolates and 94% of the typed  
247 NmW/cc11 isolates.

248 Moreover, the “South American-UK” sub-lineage can be further clustered at the genetic level  
249 into two distinct strains that corresponded to two temporal periods: “the original UK strain” with  
250 isolates from the period 2012-2016 and the “UK 2013-strain” (24) that only included isolates  
251 from the period 2015-2016 and increased significantly in 2016. Non-cc11 isolates (cc22 and  
252 other cc) did not showed significant variations during the period 2010-2016 (Fig. 4).

253

#### 254 **Epidemiological characteristics of NmW cases according to WGS data**

255 Of 132 isolates typed by WGS, 127 could be matched to clinical and epidemiological data  
256 collected through mandatory notification. We compared the characteristics of cases according to  
257 four groups defined using WGS data: the “original UK strain”, the “UK 2013-strain”, the  
258 “Anglo-French Hajj” sub-lineage and non-cc11 lineages (Table 2). There was no overall  
259 statistical difference in the distribution of cases by age group between these four groups.  
260 However a sharp increase in the number of cases due to the ‘UK 2013-strain’ was observed in  
261 2015 and 2016 in individuals aged 15 years old and over (Fig. 5). In total, there were 36 IMD

262 cases due to the “UK 2013-strain” among which 34 (94%) occurred in adults aged 15 years old  
263 and over. In the other WGS group, the number of cases in each age group was stable overall the  
264 study period, excepting the “Anglo-French Hajj” strain mainly affecting children in 2012.

265 The CFR was 25.9% among cases within the “South American-UK” sub-lineage and 11.6% in  
266 the other groups (4% for the “Anglo-French Hajj” and 15.9% for non cc11) ( $p=0.04$ ). This  
267 difference was still observed but not significant when analysing the four lineages / sub-lineages  
268 distinctly. Moreover, the CFR was not significantly different between the “original UK strain”  
269 and the “UK 2013-strain” ( $p=0.67$ ). Over the period 2010-2016, 15 deaths occurred among cases  
270 due the “South American – UK” sub-lineage: 1 death among children aged 0-14 years old (CFR  
271 12%), 3 deaths among the 15-24 years old (CFR 20%), 9 deaths among the 25-59 years old (CFR  
272 43%) and 2 deaths among individuals 60 years and over (CFR 14%).

273 There was no significant association between the infecting strain and the clinical presentation  
274 (Table 2).

275 The geographical distribution of IMD cases according to the four WGS groups is shown in Fig. 6  
276 suggesting a country-wide expansion of the strains of “South American – UK” sub-lineage in  
277 2015 and 2016. It is noteworthy that 5 cases due to these strains occurred in persons who reported  
278 a city of residence outside France: 2 cases in 2012 who lived in UK and Australia (both “original  
279 UK strain”), 2 cases in 2014 who lived in UK (1 case due to the “original UK strain” and 1 case  
280 due to the “UK 2013-strain”) and 1 case in 2015 who lived in UK (“UK 2013-strain). However,  
281 without comprehensive information about their history of travel prior to IMD, it is not possible to  
282 determine if they acquired the infection in France or in their country of residence.

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286 **Discussion**

287 Three periods with high number of NmW IMD cases were observed in France in the last  
288 17 years, considering both number of cases and their proportion relatively to all IMD cases:  
289 2000-2003, 2012 and 2016. All of them were caused by NmW/cc11 isolates. Typing analysis  
290 showed that most isolates in 2012 clustered within the “Anglo-French Hajj” sub-lineage. This is  
291 consistent with a previous report on cases imported from Sub-Saharan countries to France in  
292 2012 (25). WGS provided additional discrimination compared to the classical MLST approach  
293 and showed the emergence of the Anglo-French Hajj strain in 2012 (10). Few other isolates  
294 represented local cc11 isolates that were still clustered within the “Anglo-French Hajj” sub-  
295 lineage. These isolates may have diverged from a common ancestor introduced in France since at  
296 least the year 2000 or may have been imported later on. The last increase in the number of NmW  
297 IMD cases was observed in 2016 and was mostly due to the “UK 2013-strain”, which emerged in  
298 the UK from the “original UK strain”, a descendant from the South American strains (13, 14, 17).  
299 The strain may now to have expanded even more widely and other countries have reported  
300 increase incidence of NmW (Australia, The Netherlands and Spain,) (26-28). The “South  
301 American-UK” sub-lineage is distinct from the “Anglo-French Hajj” sub-lineage although all  
302 isolates shared the same genotypic formula (W:P1.5,2:F1-1:cc11) suggesting an important role  
303 for variation of other bacterial components (17).

304 Surveillance of IMD in France is associated with high exhaustiveness and completeness of data.  
305 In a study conducted in 2011, the exhaustiveness was estimated at 91% for mandatory  
306 notification and 84% for NRCM (data not published). Completeness of data is also very high and  
307 has improved in recent years. Since 2010, there has been less than 7% missing information for  
308 the strain group identification and less than 3% missing information on patients’ vital status  
309 resulting from IMD.

310 The CFR was higher for the “South American-UK” sub-lineage compared to the other lineages  
311 and sub-lineages which highlights the virulence of this strain as also observed in Chile and the  
312 UK (14, 29). This strain appears highly transmissible and virulent. Since its emergence in France,  
313 the “South American-UK” sub-lineage caused two clusters of cases among university students in  
314 France. In both clusters, the “UK 2013-strain” was identified. The first cluster occurred in 2016  
315 with three cases among university students in Dijon (Burgundy region). Two deaths were  
316 reported. A mass vaccination campaign was organised targeting all students and staff in the  
317 campus and no further cases have been reported. Another cluster of 2 cases (1 death) occurred in  
318 2017 among students in a small university in Paris and lead again to the organization of a  
319 vaccination campaign (30).

320 A shift in the age of NmW IMD cases has been observed since the introduction of the strains  
321 derived from “South American / UK” sub-lineage” in the population in France. In 2015 and 2016,  
322 the “UK 2013-strain” affected mainly individuals aged 15 years and over and was rare in children.  
323 This shift towards older age groups was not observed for other NmW lineages/sub-lineages  
324 during the period 2010-2016, nor in other studies on NmW previously conducted in France (31)  
325 (10). Meningococcal isolates carriage and circulation occurs most frequently among adolescents  
326 and young adults (32). The shift of IMD to older age groups was suggested as a predictor for  
327 epidemic periods of IMD (33). This may reflect the expansion of a new clone in a naïve  
328 population and/or higher transmission and virulence properties of the new clone. Similar  
329 observations were made in the UK where the increase of NmW/cc11 cases was first reported in  
330 older adults during the epidemic year 2012-2013 and then extended downwards, especially to  
331 adolescents and infants, by the epidemic year 2014/2015 (14, 34). However, in Chile where the  
332 “South American-UK” sub-lineage emerged, in 2011-2012, 47% of cases were reported in  
333 children <5 years (29). Accordingly, vaccination against NmW was initiated in October 2012 in

334 children aged 9 months to 5 years using the tetravalent conjugate vaccine. A direct effect was  
335 observed in this age group. However, this strategy did not show any impact in other age groups  
336 and it was argued that targeting adolescents and young adults may be needed to improve the  
337 vaccination strategy against NmW in Chile (12). In the UK, an adolescent program using  
338 MenACWY conjugate vaccine was recommended in 2015 targeting 13-14 years old subjects and  
339 university entrants in addition to a catch up campaign for 14-18 year olds during 2015 to 2017  
340 (35). A major decrease in the number of NmW cases was observed in the first vaccine-targeted  
341 cohort who entered university (34). The epidemiology of NmW IMD should be followed up  
342 carefully in order to guide vaccination strategies. If a vaccination was to be recommended in  
343 France, epidemiological data will be instrumental in identifying the optimal target population.  
344 Epidemiological data available for the first semester of 2017 are consistent with those presented  
345 here and show the persisting increase in the number of NmW IMD cases (32 cases in the first half  
346 of 2017 versus 24 cases in the first half of 2016), a high proportion of cases aged 15 years and  
347 over (75%) and a high CFR (31%). Finally, WGS provided new inputs in the analysis to describe  
348 the epidemiological and clinical profile of this emerging strain. We found some specific  
349 characteristics linked to the “UK 2013-strain” although comparison between lineages / sub-  
350 lineages was probably hampered by the small number of cases in each group. Our work in  
351 addition to other studies (17, 24, 36) provides evidence on the usefulness of combining WGS and  
352 epidemiological data to better describe the population expansion and spread of *N. meningitidis*  
353 and provides good basis for further work on the routine use of WGS in the surveillance of IMD in  
354 France.

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Table 1: Characteristics of invasive meningococcal disease (IMD) cases according to strain groups, France, 2015-2016 (source: mandatory notification and National reference centre for meningococci)

*NB: data are presented for the most frequent groups*

	For each group				p-value	For group W versus other groups (BCY)		
	Group B (n=503) n (%)	Group C (n=253) n (%)	Group W (n=77) n (%)	Group Y (n=116) n (%)		Group W (n=77) n (%)	Group BCY (n=872) n (%)	p-value (W vs BWY)
<b>Age group (years)</b>					<b>&lt;10<sup>-3</sup></b>			<b>0.003</b>
0-14	243 (48.3)	89 (35.2)	15 (19.5)	23 (19.8)		15 (19.5)	355 (40.7)	
15-24	100 (19.9)	54 (21.3)	21 (27.3)	20 (17.2)		21 (27.3)	174 (20.0)	
25-59	104 (20.7)	60 (23.7)	21 (27.3)	24 (20.7)		21 (27.3)	188 (21.6)	
≥ 60	56 (11.1)	50 (19.8)	20 (26.0)	49 (42.2)		20 (26.0)	155 (17.8)	
<b>No. of deaths</b>	39 (7.8)	31 (12.3)	17 (22.1)	30 (17.2)	<b>&lt;10<sup>-3</sup></b>	90 (10.4)	17 (22.1)	<b>0.002</b>
<b>Clinical presentation</b> (several sites of infection are possible)								
Meningitis	408 (81.1)	166 (65.6)	44 (57.1)	71 (61.2)	<b>&lt;10<sup>-3</sup></b>	44 (57.1)	645 (74.0)	<b>0.002</b>
Septicemia	254 (50.5)	152 (60.1)	60 (77.9)	90 (77.6)	<b>&lt;10<sup>-3</sup></b>	60 (77.9)	496 (56.9)	<b>&lt;10<sup>-3</sup></b>
Arthritis	7 (1.3)	19 (7.5)	7 (9.1)	5 (4.3)	<b>&lt;10<sup>-3</sup></b>	7 (9.1)	31 (3.6)	<b>0.01</b>
<b>Purpura fulminans</b>	113 (24.8)	56 (24.1)	8 (13.1)	9 (9.1)	<b>0.002</b>	8 (13.1)	178 (22.7)	0.08

Table 2: Characteristics of NmW invasive meningococcal disease (IMD) cases according to the four lineages/sub-lineages identified by Whole-Genome Sequencing (WGS), France, 2010-2016 (source: mandatory notification and National reference centre for meningococci)

	Original UK strain	UK 2013-strain	Anglo-French Hajj	Not cc11 lineages	p-value
	N=22 n (%)	N=36 n (%)	N=25 n (%)	N=44	
<b>Age (years)</b>					0.09
0-14	6 (27.3)	2 (5.6)	9 (36.0)	10 (22.7)	
15-24	3 (13.7)	12 (33.3)	4 (16.0)	9 (20.4)	
25-59	8 (36.4)	13 (36.1)	7 (28.0)	9 (20.4)	
≥ 60	5 (22.7)	9 (25.0)	5 (20.0)	16 (36.4)	
<b>Case fatality rate</b>	5 (22.7)	10 (27.8)	1 (4.0)	7 (15.9)	0.08
<b>Clinical presentation</b> (several sites of infection are possible)					
Meningitis	12 (54.5)	20 (55.6)	16 (64.0)	20 (45.4)	0.51
Septicemia	17 (77.3)	30 (83.3)	18 (72.0)	31 (70.4)	0.57
Arthritis	1 (4.5)	4 (11.1)	3 (12.0)	4 (9.1)	0.87
<b><i>Purpura fulminans</i></b>	3 (17.6)	4 (13.3)	2 (8.3)	5 (13.9)	0.85

Figure 1: Number of NmW invasive meningococcal disease (IMD) cases and proportion of NmW over all IMD cases, France, 2000-2016 (source: mandatory notification and National reference centre for meningococci)

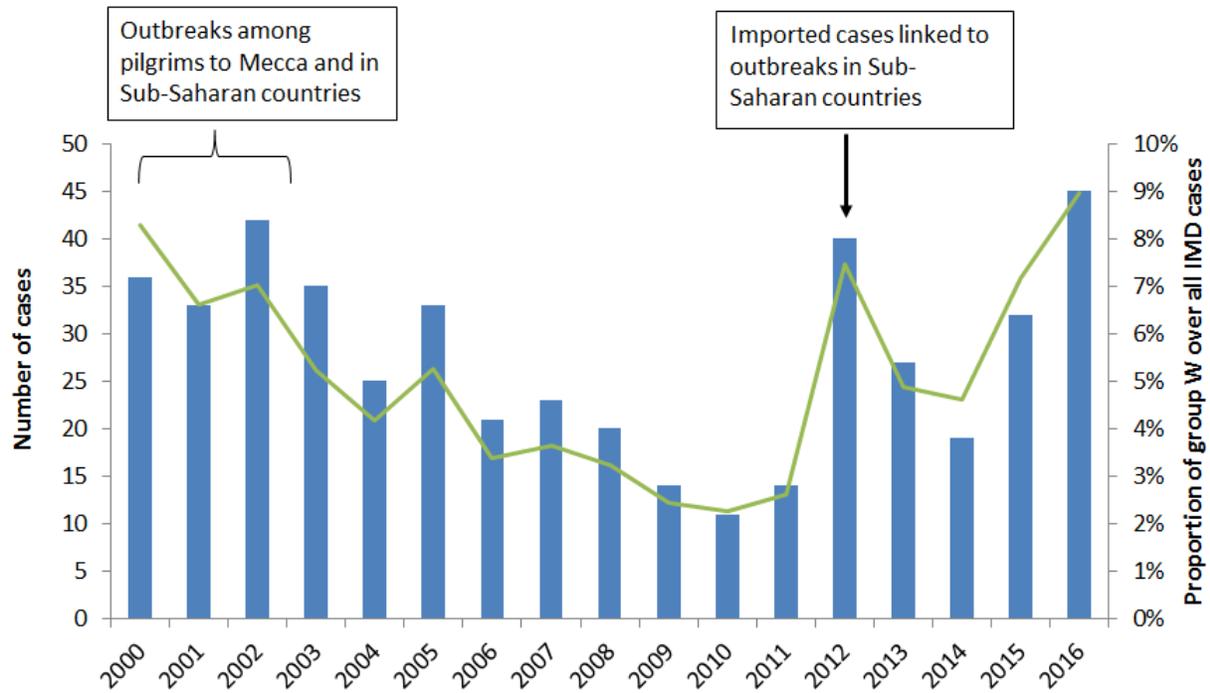


Figure 2: Annual distribution of NmW invasive meningococcal disease (IMD) corresponding to both culture- and non-culture-confirmed cases by major clonal complexes characterised by the National reference centre for meningococci (NRCM), France, 2000-2016. For the period 2000-2009, MLST typing was not systematically performed and MLST data were obtained for 52% to 100% of cases. Missing data in the period 2010-2016 correspond to non-culture cases. PCR was added in the mandatory reporting criteria in 2002, explaining the discrepancy in number of cases between figure 1 and 2 for the period 2000-2002

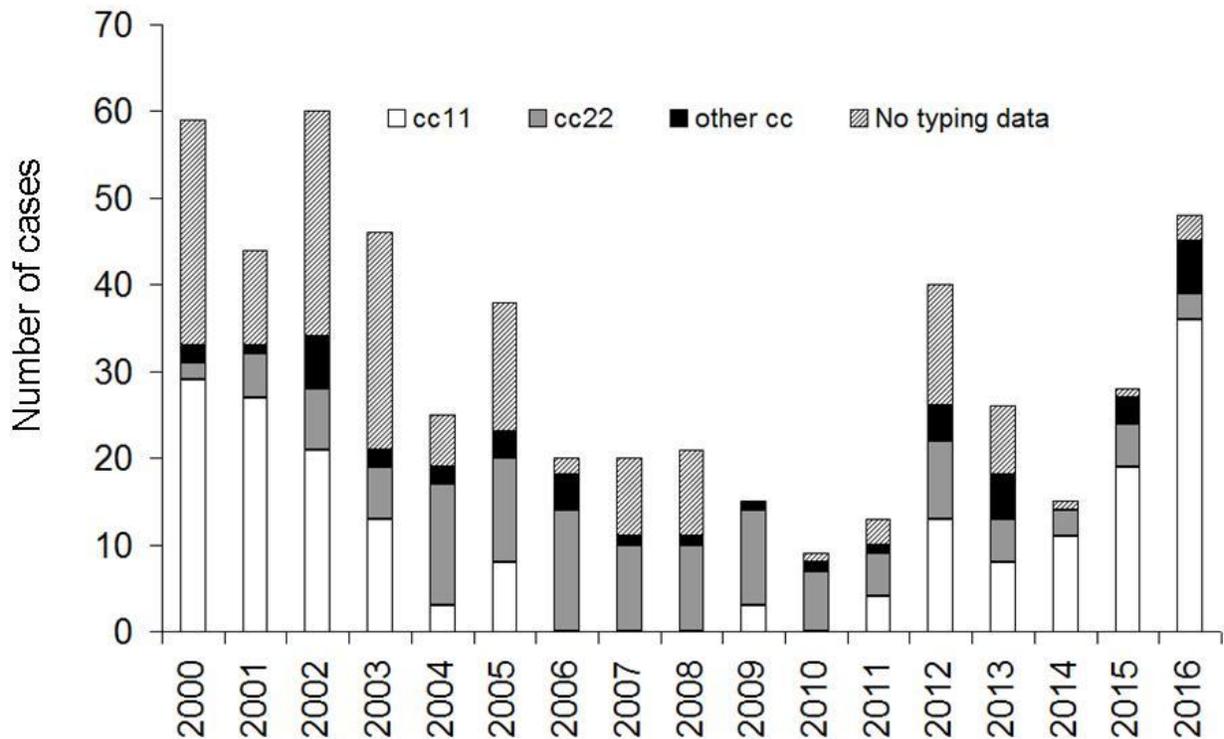


Figure 3 : Neighbour-net phylogenetic network of all invasive NmW cultured isolates received at the national reference centre for meningococci, France, 2010-2016 (A) Global view of the phylogenetic tree showing all the genetic lineages identified. In (B) and (C) a zoom on the tree that includes the non cc11 isolates (cc22 and other cc isolates). In (D) are depicted a zoom on the cc11 isolates that were resolved into the Anglo-French-Hajj sub-lineage. One isolates representative to the Hajj 2000 outbreak was included (ID31165 red arrow). The isolates that were highly related to the Hajj strain were surrounded by a solid line circle and those that were more distantly related to the Hajj strain surrounded by a dashed line circle. (E) The South American-UK sub-lineage that included the two strains: the original UK strain and the UK 2013-strain according to (24). One representative isolate (ID20196) for the “original UK strain” was indicated by a red arrow. One representative isolate (ID30167) for the “UK 2013-strain” was indicated by a blue arrow. The coloured circles represent individual isolates for each year of the period 2010-2016.

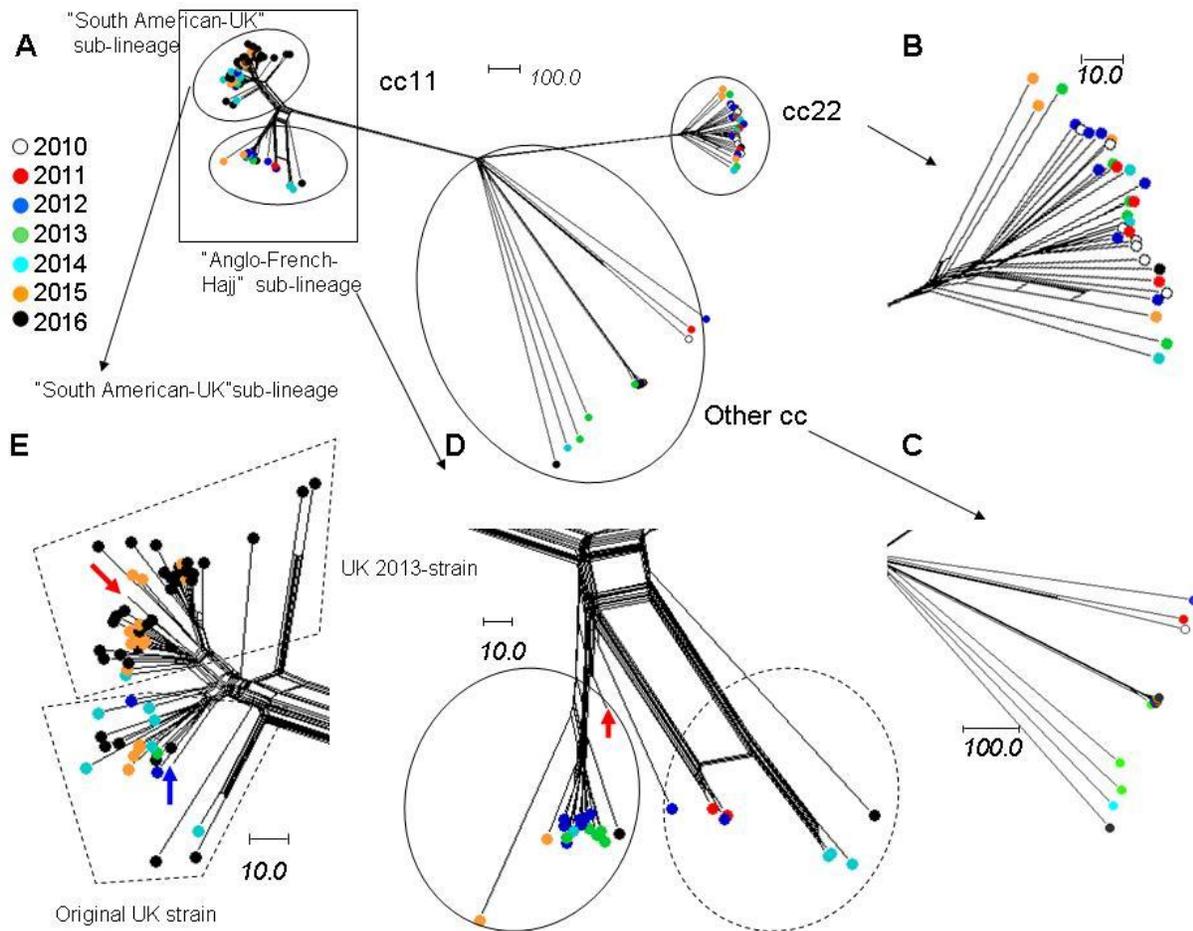


Figure 4: Annual distribution of NmW invasive meningococcal disease (IMD) isolates by WGS sub-lineage characterised by the National reference centre for meningococci, France, 2010-2016. Cases are classified in four groups using WGS data: “original UK strain”, “UK 2013-strain”, Anglo-French-Hajj and non-cc11.

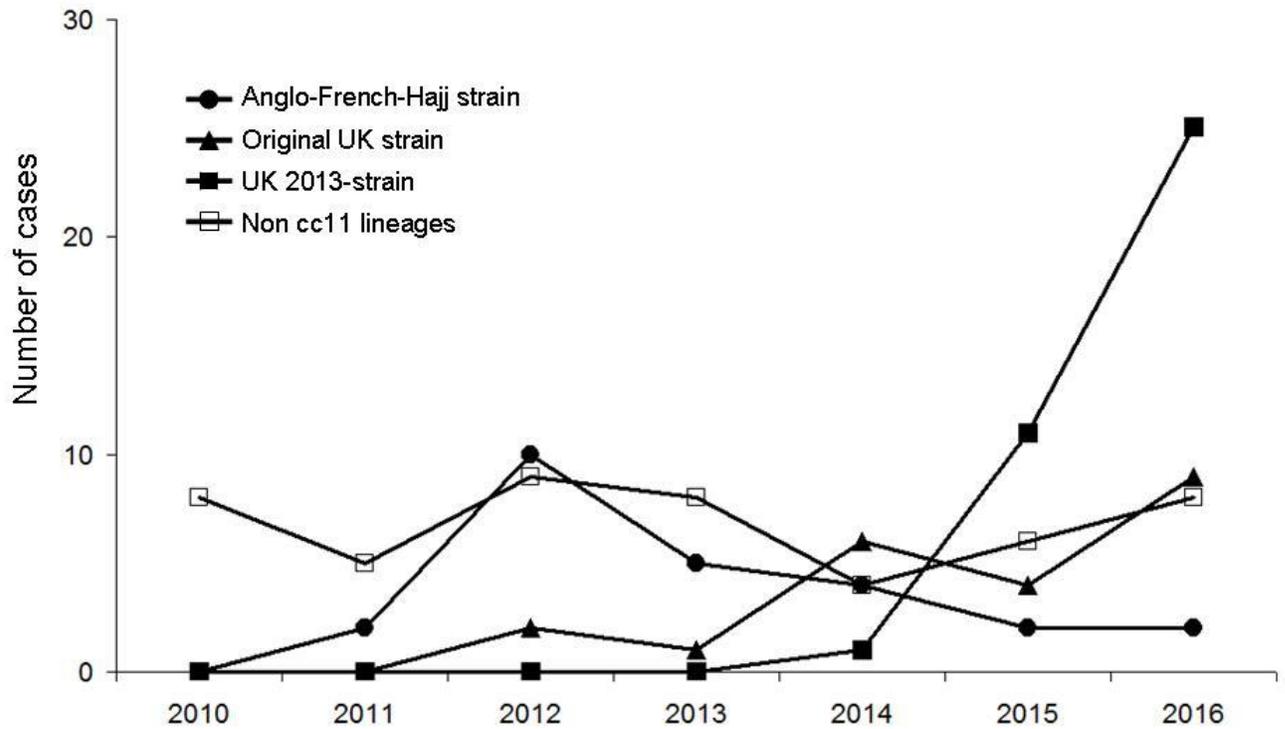


Figure 5: Number of NmW invasive meningococcal disease (IMD) cases by age group and year, according to the four lineages/sub-lineages identified by WGS, France, 2010-2016

Cases are classified in four groups using WGS data: “original UK strain”, “UK 2013-strain”, Anglo-French-Hajj and non-cc11.

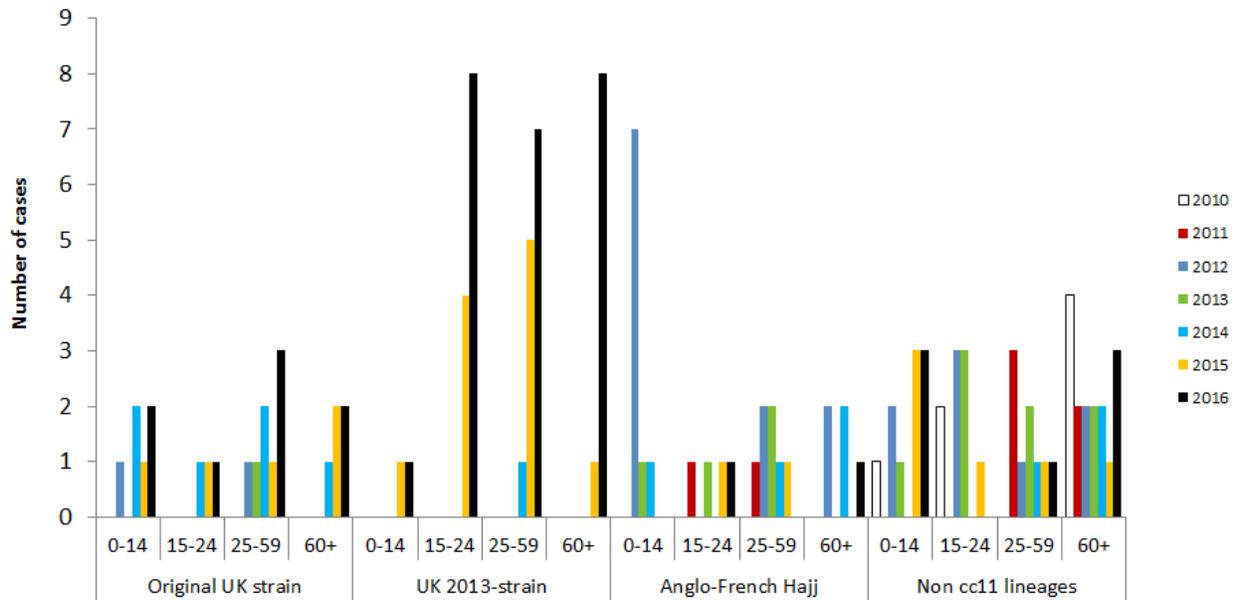


Figure 6

Geographic distribution of NmW invasive meningococcal disease (IMD) cases according to their place of residence, France, 2010-2016.

Cases are classified in four groups using WGS data: “original UK strain”, “UK 2013-strain”, Anglo-French-Hajj and non-cc11.

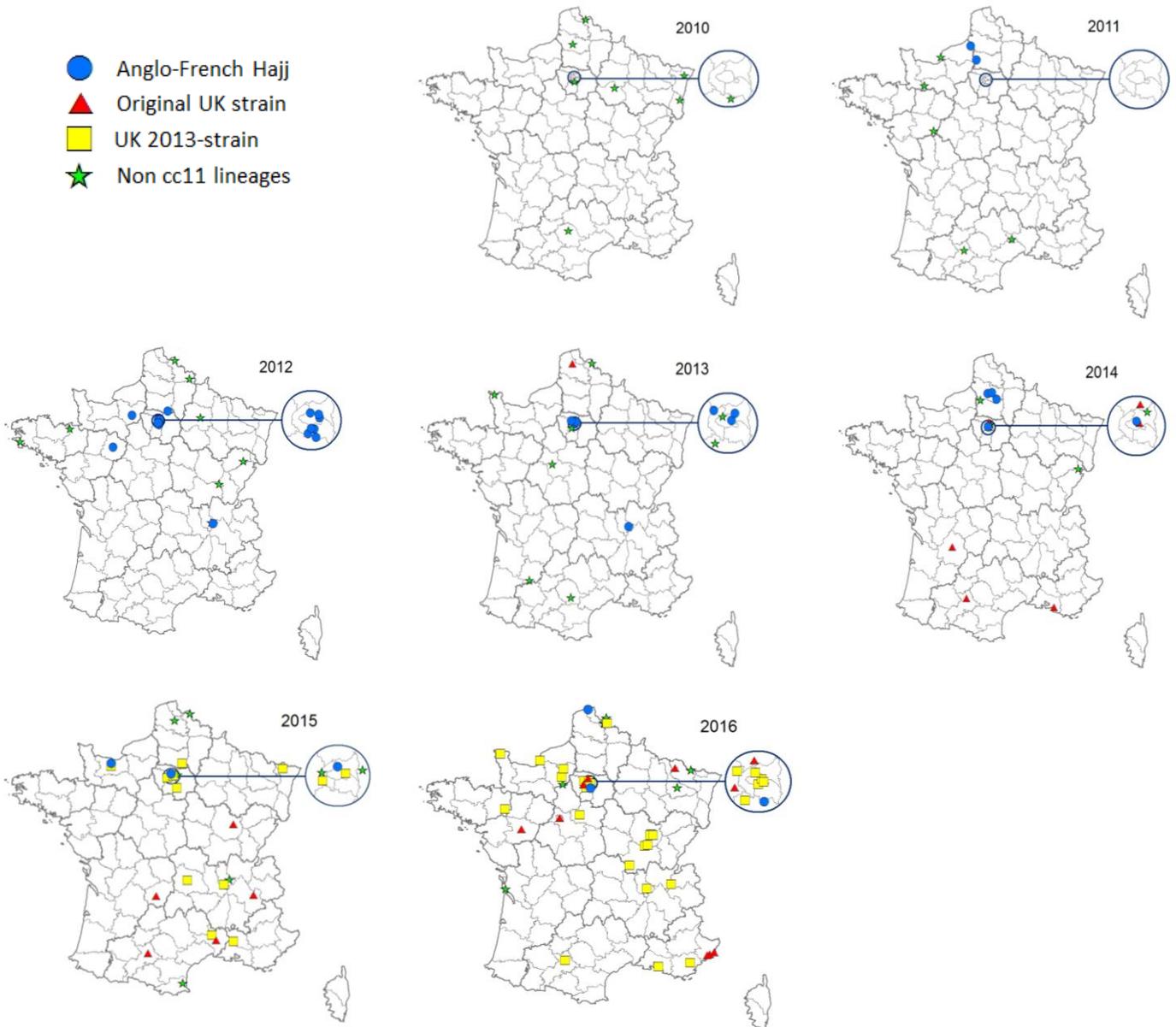


Table 1: Characteristics of invasive meningococcal disease (IMD) cases according to strain groups, France, 2015-2016 (source: mandatory notification and National reference centre for meningococci)

*NB: data are presented for the most frequent groups*

	For each group				p-value	For group W versus other groups (BCY)		
	Group B (n=503) n (%)	Group C (n=253) n (%)	Group W (n=77) n (%)	Group Y (n=116) n (%)		Group W (n=77) n (%)	Group BCY (n=872) n (%)	p-value (W vs BWY)
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<b>No. of deaths</b>	39 (7.8)	31 (12.3)	17 (22.1)	30 (17.2)	<b>&lt;10<sup>-3</sup></b>	90 (10.4)	17 (22.1)	<b>0.002</b>
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Arthritis	7 (1.3)	19 (7.5)	7 (9.1)	5 (4.3)	<b>&lt;10<sup>-3</sup></b>	7 (9.1)	31 (3.6)	<b>0.01</b>
<b>Purpura fulminans</b>	113 (24.8)	56 (24.1)	8 (13.1)	9 (9.1)	<b>0.002</b>	8 (13.1)	178 (22.7)	0.08

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<b><i>Purpura fulminans</i></b>	3 (17.6)	4 (13.3)	2 (8.3)	5 (13.9)	0.85

Figure1

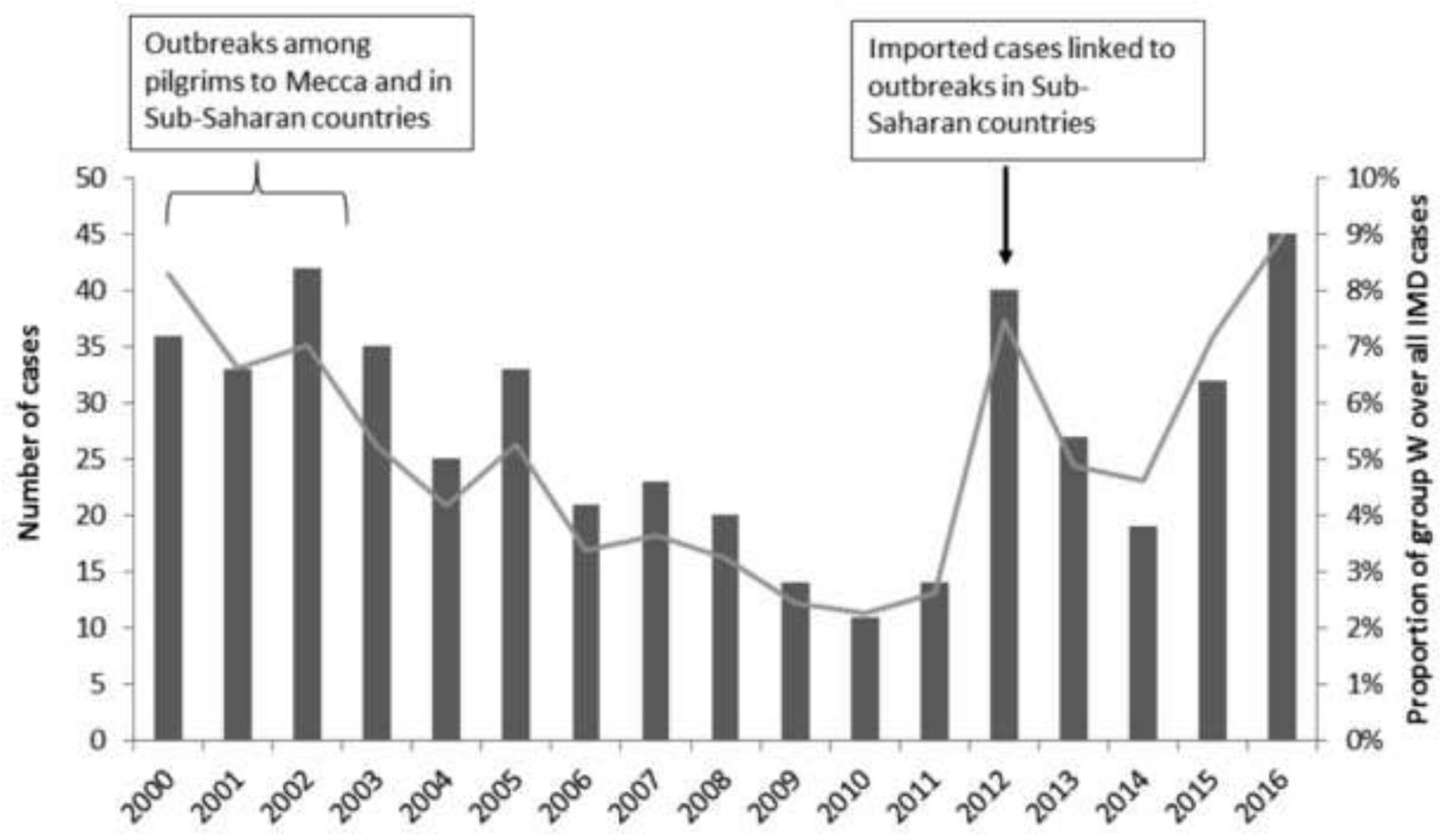


Figure2

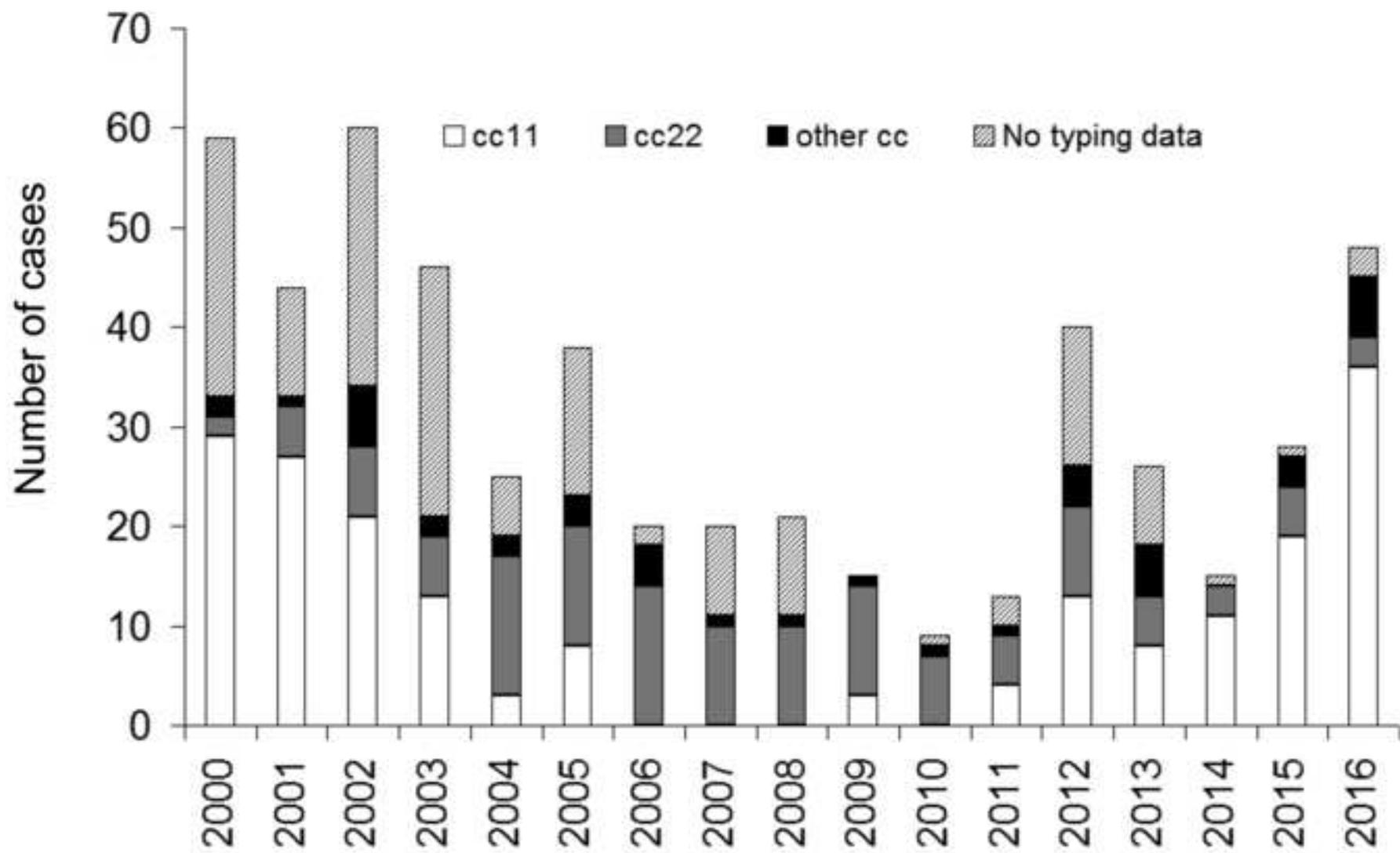


Figure3

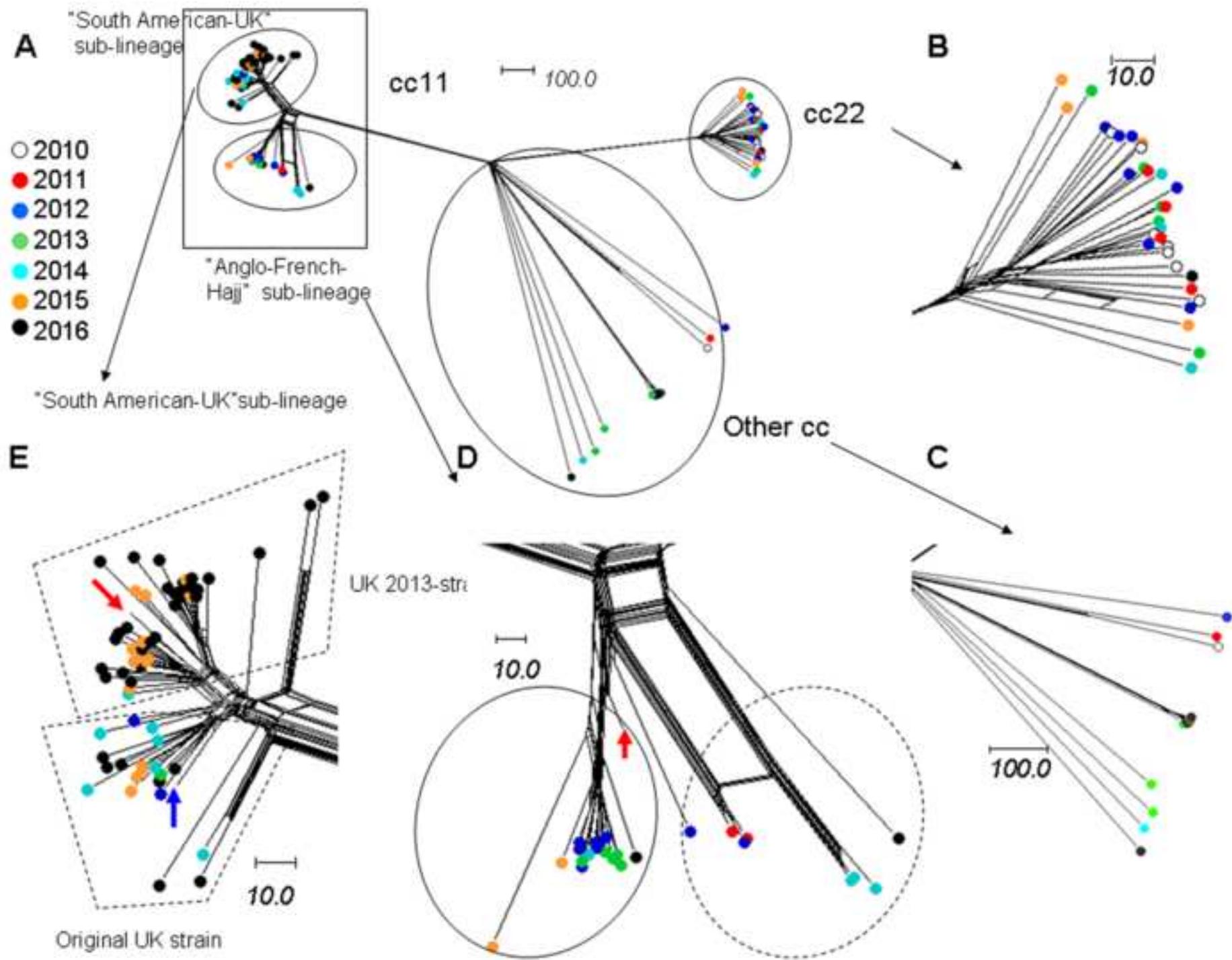


Figure4

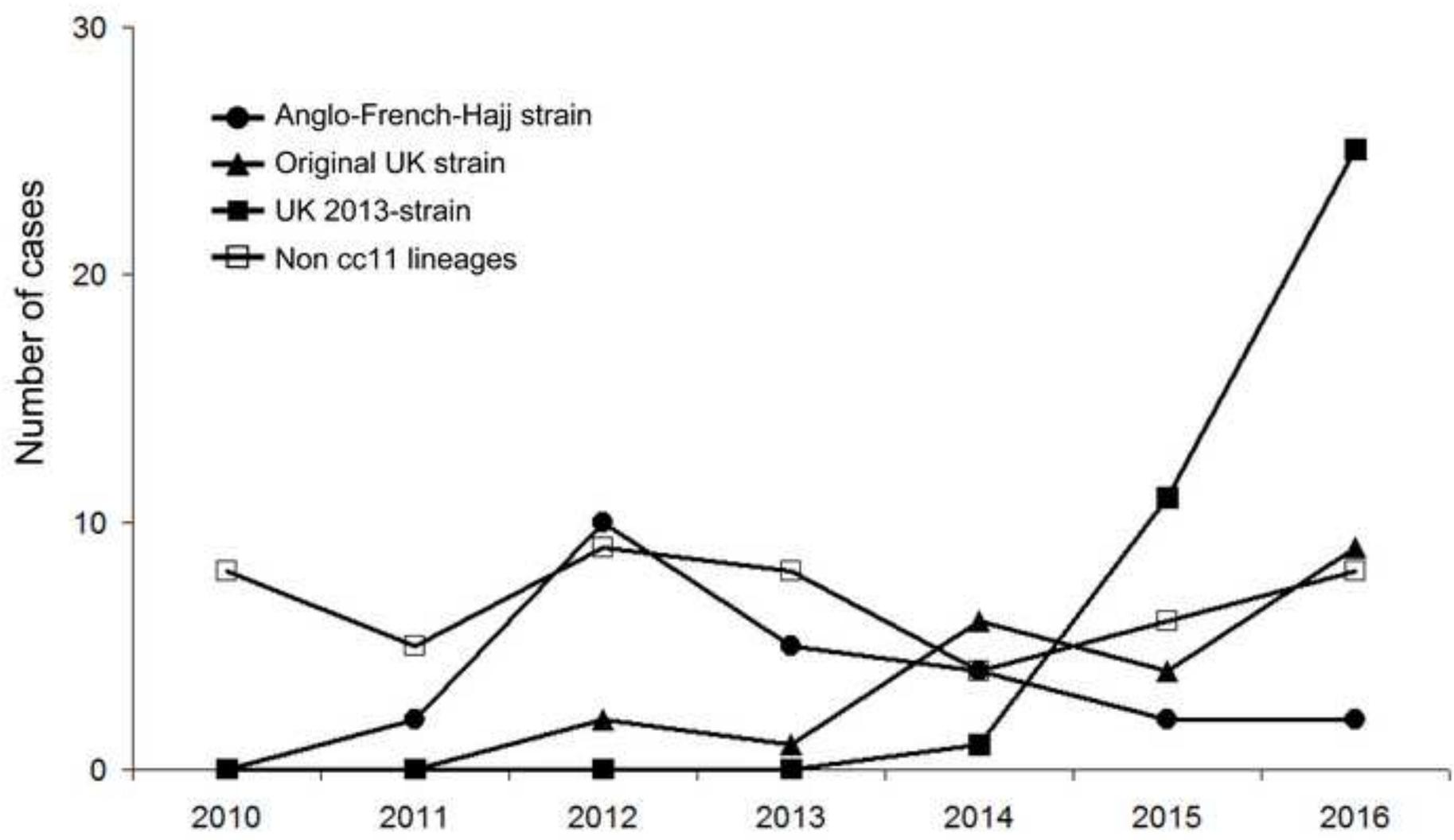


Figure5

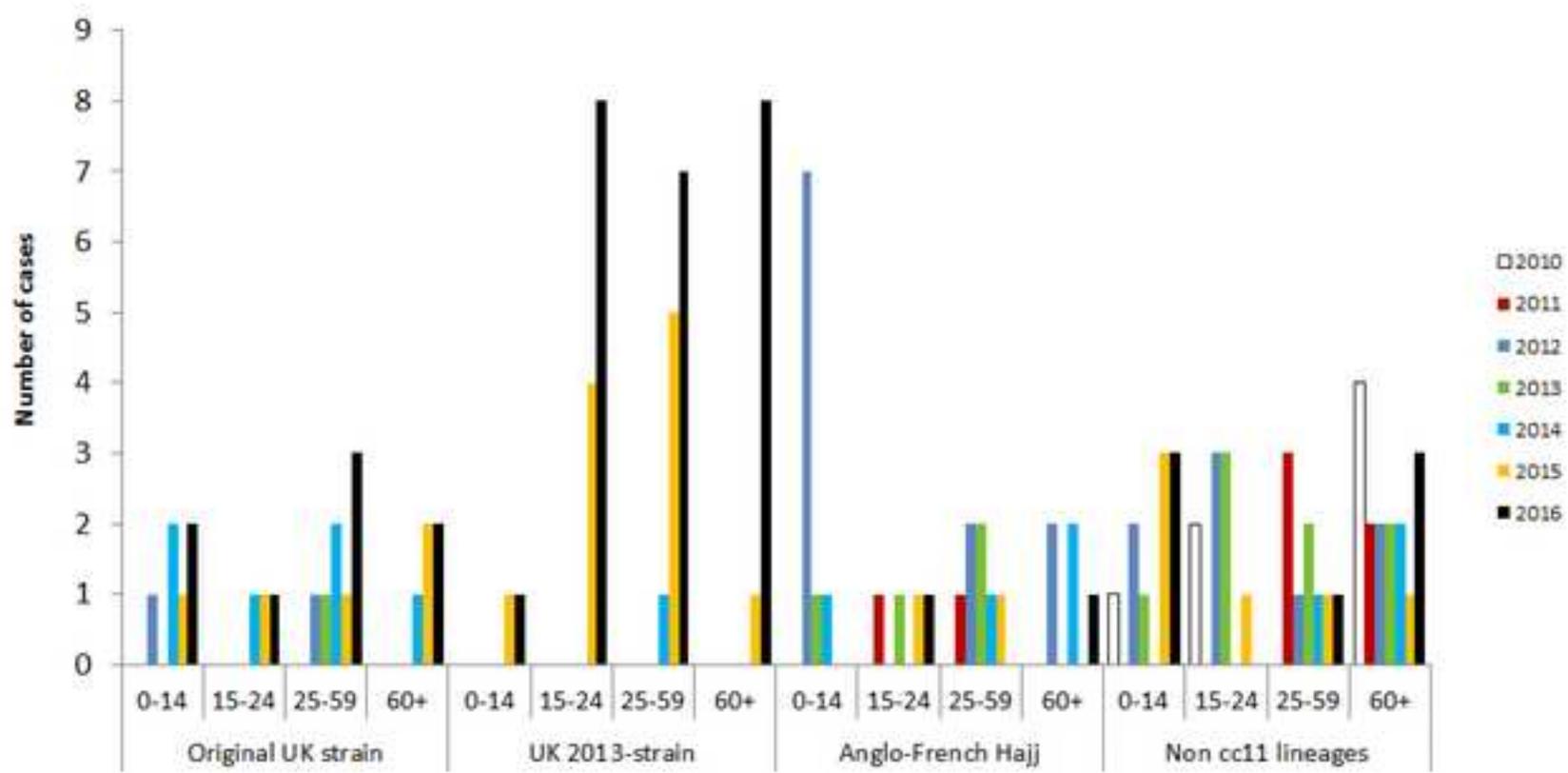
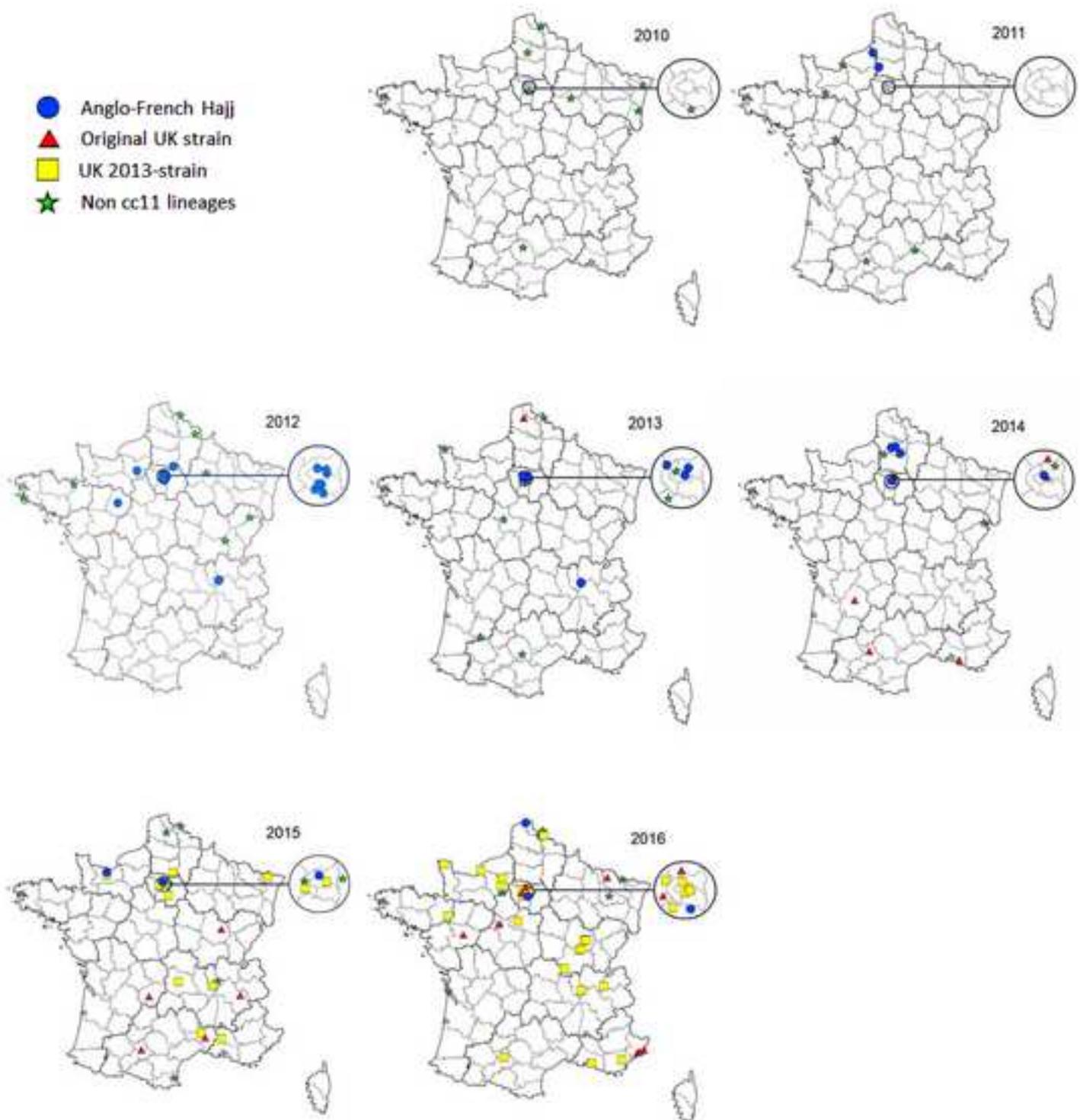


Figure6



**Supplementary Table**

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