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1 **Virological and epidemiological patterns of swine influenza A virus infections in**
2 **France: cumulative data from the RESAVIP surveillance network, 2011–2018**

3
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29 **Abstract**

30 Swine influenza A viruses (swIAVs) cause acute respiratory syndromes in pigs and may also
31 infect humans. Following the 2009 pandemic, a network was established in France to
32 reinforce swIAV monitoring. This study reports virological and epidemiological data
33 accumulated through passive surveillance conducted during 1,825 herd visits from 2011 to
34 2018. Among them, 887 (48.6%) tested swIAV-positive. The proportion of positive cases
35 remained stable year-on-year and year-round. The European avian-like swine H1N1 (H1_{av}N1)
36 virus was the most frequently identified (69.6%), and was widespread across the country. The
37 European human-like reassortant swine H1N2 (H1_{hu}N2) virus accounted for 22.1% and was
38 only identified in the north-western quarter and recently in the far north. The 2009 pandemic
39 H1N1 (H1N1_{pdm}) virus (3.6%) was detected throughout the country, without settling in areas
40 of higher pig densities. Its proportion increased in winter, during the seasonal epidemics in
41 humans. The European human-like reassortant swine H3N2 as well as H1_{av}N2 viruses were
42 identified sporadically. In up to 30% of swIAV-positive cases, pigs exhibited clinical signs of
43 high intensity, regardless of the viral subtype and vaccination program. The recurrent pattern
44 of the disease, i.e., an endemic infection at the herd level, was reported in 41% of cases and
45 mainly affected post-weaning piglets (OR=5.11 [3.36-7.76]). Interestingly, the study also
46 revealed a significant association between the recurrent pattern and sow vaccination
47 (OR=1.96 [1.37-2.80]). Although restricted to the studied pig population, these results bring
48 new knowledge about swIAV dynamics and infection patterns in pig herds in France.

49 **Highlights**

- 50 • The H1_{av}N1 virus was the major European swIAV identified in France
- 51 • The H1_{hu}N2 virus was the second most detected proportionally
- 52 • H1N1pdm proportion was higher in winter and in areas with smallest pig populations
- 53 • Post-weaning piglets were more frequently affected by recurrent pattern of influenza
- 54 • Recurrent infections were significantly associated to vaccination of sows

55

56 **Keywords:** Pigs, Swine Influenza A Virus, Surveillance, RESAVIP, Epidemiological features

57 **Introduction**

58 Swine Influenza A virus (swIAV) infections in pigs are responsible for acute respiratory
59 syndromes usually characterized by cough, sneezing, nasal discharge, fever and apathy, and
60 usually last 5–7 days (Janke, 2013). The disease may occasionally be more severe and can be
61 associated with growth retardation and/or mortality. Symptoms of chronic respiratory disease
62 may also be observed, particularly in the context of the porcine respiratory disease complex
63 (PRDC) (Vincent et al., 2017). In addition to episodic swIAV infections, recurrent influenza
64 outbreaks have been described in pig herds in France, i.e., swIAV infections that affect
65 successive batches of pigs at a given physiological stage (Rose et al., 2013). These recurrent
66 outbreaks have been shown to be associated with endemic persistence of swIAV infections at
67 the herd level (Ferreira et al., 2017; Rose et al., 2013). As a result, alongside their impact on
68 animal welfare, swIAV infections cause significant economic losses for the pig industry
69 (Bennett and IJpelaar, 2005; Brons et al., 2011).

70 SwIAVs may also be transmitted to humans where they can cause influenza and have
71 sometimes led to epidemics. In 2009, a novel H1N1 virus of swine origin emerged in humans
72 and was responsible for the first influenza pandemic of the 21st century, before becoming
73 seasonal in humans as well as enzootic in pig populations worldwide after reverse zoonosis
74 (Adlhoch et al., 2014; Simon et al., 2014). Following the 2009 pandemic, research teams,
75 expert laboratories, and international organizations (WHO/OIE/FAO) launched a request to
76 reinforce swIAV surveillance in order to better understand the emergence of new viruses
77 (Vincent et al., 2014). In Europe, the European Surveillance Network for Influenza in Pigs
78 (ESNIP) showed that viruses from three subtypes (HxNy) and four genetic lineages, defined
79 by the nature and origin of the genes encoding the hemagglutinin (HA) and neuraminidase
80 (NA) surface glycoproteins, were circulating enzootically in the European pig population

81 (Simon et al., 2014). These predominant lineages were the European avian-like swine H1N1
82 (H1_{av}N1), the pandemic-like swine H1N1 (H1N1pdm), the European human-like reassortant
83 swine H1N2 (H1_{hu}N2), and the European human-like reassortant swine H3N2 (H3N2). They
84 were detected with variable frequencies depending on the geographical area. Their co-
85 circulation induced reassortant viruses through genomic reassortment, i.e., exchange of one or
86 several genomic segments between different viruses through coinfection events, among which
87 some became enzootic in certain countries (Watson et al., 2015). Moreover, mutations in the
88 HA and/or NA genes can lead to the production of variants that are antigenically distant from
89 their parental viruses, contributing to the emergence of new swIAVs. As an example, a novel
90 variant called H1_{hu}N2 Δ ₁₄₆₋₁₄₇ (H1_{hu}N2v) was detected in France in 2012 following genetic and
91 antigenic drift in the parental H1_{hu}N2 lineage (Bonin et al., 2018).

92 After the 2009 pandemic, French stakeholders in swine health worked jointly to create a
93 national public-private network for swIAV surveillance (*Réseau national de Surveillance des*
94 *Virus Influenza A chez le Porc*; RESAVIP) and monitor swIAVs in circulation on farms
95 despite their non-regulated health hazard (Garin et al., 2017; Hervé et al., 2014). The
96 RESAVIP network was therefore set up in April 2011 in order: i) to identify the swIAVs
97 causing respiratory outbreaks in pigs, ii) to assess their distribution across the country, and iii)
98 to better understand viral strain dynamics and the epidemiological patterns of swIAV
99 infections.

100 In this article, we report on the diversity and the temporal and spatial distribution of the
101 various swIAVs that were identified in pig herds in France from April 2011 to March 2018 in
102 the framework of the RESAVIP network. To show possible associations between clinical
103 characteristics, virus lineages, and/or epidemiological variables, we analysed statistically the
104 data collected from the farms sampled during this 7-year period.

105

106 **Materials & Methods**

107 **The RESAVIP network**

108 RESAVIP is coordinated by the French Federation of agricultural and agro-food cooperatives
109 (*Coop de France*). The other national partners are the French Directorate general for food
110 (*Direction Générale de l'Alimentation*; DGAL), the French Agency for food, environmental
111 and occupational health & safety (*Agence Nationale de Sécurité Sanitaire de l'alimentation,*
112 *de l'environnement et du travail*; ANSES), the French society of veterinary technical groups
113 (*Société Nationale des Groupements Techniques Vétérinaires*; SNGTV), the French
114 Association of directors and executives of public veterinary laboratories (*Association*
115 *française des Directeurs et cadres de Laboratoires Vétérinaires publics d'Analyses*;
116 ADILVA), the French Federation of health protection groups (*Fédération nationale des*
117 *Groupements de Défense Sanitaire*; GDS France) in the frame of the Epidemiological
118 surveillance platform for animal health (*Plateforme d'Epidémiosurveillance en Santé*
119 *Animale*; ESA Platform). The surveillance is conducted in mainland France and is carried out
120 at the administrative region level with oversight by regional coordinators. It is based on
121 sampling and description of pigs showing influenza-like illness (ILI) by volunteer pig
122 veterinarians in conjunction with the farmer, followed by swIAV detection by local accredited
123 laboratories, and virus identification by the National Reference Laboratory (NRL) for swine
124 influenza. The studied population therefore only includes pig herds that exhibited ILI and that
125 have been sampled by a volunteer veterinarian. The anonymity of farms and farmers is
126 preserved.

127 **Case definition, biological samples and epidemiological data collection**

128 Biological samples were taken from pigs exhibiting ILI by a volunteer veterinarian, either
129 after a call from a farmer to report an outbreak, or during a previously planned herd visit. In

130 this study, all visits were considered to be independent of each other, even if some herds
131 could have been visited more than once during the 7-year monitoring. In each affected herd,
132 nasal swabs (Sigma Virocult[®], Kitvia, Labarthe-Inard, France) were collected from three
133 growing pigs of the same age, or three breeders, that were selected based on their rectal
134 temperature (above 40.5°C preferentially). This constituted a suspected case. The case was
135 considered to be swIAV-positive if at least one of the three samples tested positive in the first-
136 line laboratory detection test (see below).

137 In addition to biological samples, the veterinarian collected outbreak-related information on a
138 specific accompanying datasheet (SAD), asking for information about the sampling context
139 and date, the geographic location of the farm at the French administrative region level
140 (administrative area of 6,130 km² on average), the herd type, the vaccination program, the age
141 and/or physiological stage of the sampled animals, the individual rectal temperatures, the
142 medication applied, the ILI intensity, and the epidemiological pattern of the outbreak (see
143 details in Appendix 1).

144 **swIAV detection and subtyping**

145 Nasal swab supernatants were submitted to accredited local public veterinary laboratories for
146 swIAV M gene real-time RT-PCR (RT-qPCR) using commercial kits previously validated by
147 the NRL, LSI VetMAX Swine Influenza A -A/H1N1/2009 included (LSI-Life Technologies,
148 Lissieu, France) or Adiavet SIV Real Time (ADIAGENE-BioX Diagnostics, Ploufragan,
149 France) (Pol et al., 2011). The analytical specificity and sensitivity of both tests were 100%
150 each. All M gene-positive samples (Cq values <45), as well as corresponding viral RNA
151 extracts, were sent to the NRL for subsequent subtyping using RT-qPCR assays that
152 specifically amplify and discriminate the HA and NA genes from the different lineages of
153 swIAVs known to be enzootic in European pig herds (H1_{av}N1, H1_{hu}N2, H3N2, and

154 H1N1pdm). Briefly, three in-house RT-qPCR assays allowing the amplification of the H1_{av},
155 H1_{hu} and H3 genes, respectively, and one duplex RT-qPCR for the detection of the N1 and N2
156 genes were applied, as previously described (Bonin et al., 2018). The N1 RT-qPCR amplifies
157 NA genes from both the H1_{av}N1 and the H1N1pdm lineages, whereas the N2 RT-qPCR
158 amplifies NA genes from both the H1_{hu}N2 and the H3N2 lineages. When an H1_{hu}Ny virus
159 was detected, another RT-qPCR was run that specifically targets an H1_{hu} variant gene
160 (H1_{hu}V), which was first detected in France in 2012 (Bonin et al., 2018). RT-qPCRs for the
161 amplification of the H1pdm and N1pdm genes were carried out using commercial kits
162 previously validated by the NRL, Adiavet A/H1N1 2009 Real Time (ADIAGENE-BioX
163 Diagnostics, Ploufragan, France) or LSI VetMAX Swine Influenza A/H1N1/2009-H1
164 Detection for H1pdm, and LSI VetMAX Swine Influenza A/H1N1/2009-N1 Detection (LSI-
165 Life Technologies, Lissieu, France) for N1pdm (Pol et al., 2011). Due to the lower sensitivity
166 of in-house subtyping methods as compared to the swIAV detection method, the HA- and
167 NA-gene RT-qPCRs were only run on viral RNA extracts exhibiting Cq values <35 in M
168 gene RT-qPCR (Bonin et al., 2018). When HA and/or NA gene identification was
169 unsuccessful, but the initial M gene RT-qPCR Cq value was <30, the virus detected in nasal
170 swab supernatant was propagated on MDCK cells (WHO, 2002), and further subtyping
171 analyses were performed on the isolated strain.

172 **Epidemiological data analyses**

173 The epidata reported on SADs were collected by RESAVIP regional coordinators and entered
174 into regional databases that were integrated every quarter into a national database managed by
175 the national coordinator. The temporal and spatial distributions of the different swIAV
176 lineages were collated from the collected epidata and results of lab analyses. Three outcomes
177 were also investigated in this study: (1) case positivity to swIAVs, (2) severity of clinical
178 signs (moderate or high), and (3) epidemiological patterns of swIAV infection (classical or

179 recurrent). The classic form is defined by a sporadic acute infection and the recurrent form is
180 related to an endemic pattern of infection at the herd level, affecting each successive batch of
181 pigs at a given physiological stage.

182 The statistical associations between these outcome variables and the following characteristics
183 were investigated: i) the seasonality, by distinguishing between the period of time during
184 which the seasonal influenza epidemic takes place in humans (December to March) and the
185 period outside the seasonal influenza epidemic (April to November) ii) the pig population size
186 recorded in 2010 in each administrative region according to three classes, i.e., regions housing
187 fewer than 150,000 pigs (small population), those housing from 150,000 to 500,000 pigs
188 (medium-size population), and those with 500,000 pigs or more (large population), iii) the
189 different viral subtypes (HxNy), iv) the presence or absence of breeders on site, v) the
190 application or not of a vaccination program, vi) the type of sampled animal (breeder or
191 growing pig), as well as the age of the growing pigs by distinguishing 4 classes (≤ 6 weeks of
192 age,]6–9] weeks,]9–12] weeks, or above 12 weeks of age), and vii) rectal temperature below
193 or above 40.5°C.

194 Differences between years were tested by a Pearson Chi-squared test. When the conditions
195 were not met for a Pearson Chi-squared test, a Fisher exact test was used. *P*-values < 0.05
196 were considered significant. In order to evaluate the influence of potential explanatory
197 variables on each outcome, a logistic regression model based on generalized estimating
198 equations (GEEs), to account for clustered observations from the same year (univariate
199 analysis), was implemented (Liang and Zeger, 1986). Then, variables significant at a 0.20
200 level were included in a multivariate model to account for confounders, and only variables
201 remaining significant at a 5% level, after a backward procedure, were retained in the final
202 model (Hosmer and Lemeshow, 1989).

203

204 **Results**

205 **Temporal and spatial distribution of the pig farms included in RESAVIP**

206 From April 2011 to March 2018, the RESAVIP network investigated 1,825 ILI cases (261
207 farm visits per year on average, min 244, max 309) (Table 1). The completeness of the data
208 collected in SADs varied from 78.7 to 100%, depending on the variables. In 80% of cases, the
209 herd was included in RESAVIP after the farmer called the veterinarian to report a respiratory
210 outbreak. The remaining 20% of veterinary visits were already scheduled. Importantly, nearly
211 55% of the farm visits (739/1,351) took place less than 72 hours after the first ILI clinical
212 signs were observed by the farmer. However, ILI started 3 to 7 days before the sampling date
213 in almost 34% of cases (458/1,351), and more than one week prior to sampling in just over 11
214 % of the visits (154/1,351). The frequency of swIAV-positive herds decreased as the time
215 between the onset of clinical signs and sampling increased (51.8%, 45.0% and 32.5% when
216 sampling took place less than 3 days, between 3 and 7 days, or more than 7 days after ILI
217 occurrence, respectively, $p < 0.001$), but remained relatively stable over the years, ranging
218 from 41.8 to 53.6% ($p = 0.20$) (Table 1). The swIAV genome was detected in the three
219 collected nasal swabs in three quarters of the positive cases (data not shown). Positive and
220 negative herds were reported every month during the study period (Fig. 1), showing that
221 swIAV infections as well as ILI due to other respiratory pathogens occurred throughout the
222 year, with similar monthly distributions ($p = 0.43$). Also, the proportion of herds that were
223 detected swIAV positive in winter, i.e., from December to March when the seasonal epidemic
224 occurs in humans, was not different to that of positive herds sampled from April to
225 November: 47.1% versus 49.5% ($p = 0.53$) (Table 2).

226 The visited herds were located in 51 administrative regions out of 96 (53%) in mainland
227 France, across the country (Fig. 2). At least one swIAV-positive herd was detected in 35 out
228 of these 51 administrative regions across the territory (Fig. 2). The proportion of swIAV-

229 positive herds was significantly higher in administrative regions with the largest pig
230 population size than in those with a medium-sized population (50.2% versus 42.1%, $p=0.01$)
231 (Table 2).

232 **Relative frequencies and temporal and spatial distribution of virus subtypes**

233 On average every year, 74.7% ($\pm 3.3\%$) of the viruses detected by M gene RT-qPCR were
234 identified. From the 669 swIAVs that were subtyped over the study period, H1_{av}N1 viruses
235 were the most commonly identified, accounting for 69.6% (466/669) of virus strains, with
236 annual figures ranging from 64.2% in 2015 to 80.2% in 2012 (Fig. 3). H1_{av}N1 viruses were
237 detected every year and their proportion among the swIAV-positive herds was significantly
238 lower in winter, i.e., from December to March (46.6% versus 55.7%, $p=0.002$). H1_{av}N1
239 viruses were detected in herds located in 27 administrative regions across France, regardless
240 of the pig population size ($p=0.84$) (Fig.4).

241 Other viruses of the H1N1 subtype belonged to the H1N1pdm lineage. Since RESAVIP was
242 set up, this lineage has been identified each year except in 2012, accounting for 3.6% (24/669)
243 of the subtyped viruses, with annual proportions varying from 1.3% (1/74) in 2017 to 8.2%
244 (8/98) in 2016 (Fig. 3). Since April 2011, 16/24 (66.7%) H1N1pdm strains were detected
245 from months December to March, leading to a significantly higher proportion of H1N1pdm
246 strains in winter, i.e. during the seasonal epidemic in humans (3.9% versus 1.4%, $p<0.001$). In
247 January to March 2018, they even accounted for 20% of the virus strains (6/30) identified by
248 RESAVIP during this quarter. The H1N1pdm virus was identified in 16 administrative
249 regions distributed from the North to the South of the country (Fig. 4). However, its
250 proportion among swIAVs was higher in administrative regions with small pig population
251 sizes (25%) than in those with a medium-sized (6.9%) or large population (0.6%) ($p<0.001$).

252 The second most frequent lineage was H1_{hu}N2, which accounted for 22.1% (148/669) of the
253 viruses identified during the 7 year-study period (Fig. 3). Until May 2016, H1_{hu}N2 strains
254 were only detected in the north-western part of France, but since then they have also been
255 identified in two northern administrative regions (Fig. 4). The annual proportion of H1_{hu}N2v
256 strains among the H1_{hu}N2 viruses identified in the country increased from 25% (5/20) in 2012
257 to 68.4% (13/19) in 2014 (Fig. 3). This variant was even the only representative of the
258 H1_{hu}N2 subtype detected in two north-western administrative regions. Since 2015, the
259 proportion of H1_{hu}N2v has decreased, but it was still detected in 2017. The proportion of
260 H1_{hu}N2 viruses among swIAVs was higher from December to March (14.9% versus 9.2%,
261 $p=0.003$) and was higher in administrative regions with a large pig population size than in
262 those with a medium-sized (13.2% versus 4.6%, $p<0.001$).

263 From 2014 to 2016, H3N2 viruses were detected occasionally in northern administrative
264 regions (Fig. 4). The very low number of H3N2-infected herds did not enable us to evaluate
265 any potential statistical link whether between this subtype and the season, or between this
266 subtype and the pig population size.

267 Second-generation reassortant viruses, i.e., viruses exhibiting HA and NA genes originating
268 from two distinct enzootic swIAV lineages, were also identified during the study period.
269 H1_{av}N2 viruses were identified twice (2/49) in 2011, once (1/107) in 2013, and once (1/101)
270 in 2014, in the north-western part of France where the H1_{av}N1 and H1_{hu}N2 enzootic lineages
271 were both frequently identified. In 2015, these H1_{av}N2 reassortants were detected on four
272 (4/109) occasions in the south-western region where no HxN2 enzootic viruses were detected
273 before. In 2016, one (1/98) H1_{av}N2 virus was identified in the North, where enzootic H1_{av}N1
274 and H3N2 viruses were also detected.

275 Molecular subtyping also demonstrated 18 (2.7%) herds for which nasal swab supernatants
276 contained mixtures of HA and/or NA genes from several swIAV lineages, illustrating co-
277 infection events (Fig. 3). Among them, 13 herds exhibited HA and/or NA genes from both the
278 H1_{av}N1 and H1_{hu}N2 lineages and were detected in the north-western region and in the North
279 of France. The five other herds were distributed across the country and involved HA and/or
280 NA genes from the H1N1pdm lineage together with either the H1_{av}N1 or the H1_{hu}N2 lineage
281 (Fig. 4).

282 Around a quarter of detected swIAVs (218/887) remained partially subtyped (HxN? or H?Ny)
283 or could not be identified (H?N?). Around 60% of these samples with unknown viruses
284 (131/218) were automatically excluded from subtyping analyses because exhibiting M gene
285 Cq-values >35, that indicated an apparent very low genomic load. Among other unknown
286 viruses, 31% (68/218) had a 30 < Cq-value < 35 (data not shown). Finally, only 9% (19/218) of
287 unknown viruses had a M-gene Cq < 30. In these cases, subtyping failure could have been due
288 to bad virus genome integrity or lack of HA and/or NA RT-qPCR specificity, as only one
289 mutation in primers or probe binding regions may prevent amplification.

290 **Characteristics of the sampled pig population**

291 Many questions about the visited herds and the sampled pigs were asked in the SADs. Some
292 new items were added subsequently and were therefore included in the SAD only from 2013
293 onwards; they accumulated less data than other items (Tables 1–5). All pig production types
294 encountered in France were sampled (data not shown), but 76.6% of visited farms (1,395
295 cases out of 1,821) were farrow-to-finish herds. SwIAV-positive pigs were detected in all
296 herd types, regardless of the presence or absence of breeders on site ($p=0.27$) (Table 2).

297 During the study period, only one inactivated trivalent vaccine directed against the three
298 enzootic European lineages H1_{av}N1, H3N2, and H1_{hu}N2 (Respiporc Flu[®]3, IDT Biologika

299 GmbH, Dessau-Rosslau, Germany) was authorized in France. Within the RESAVIP
300 framework, it was applied to breeders exclusively. Thus, vaccination was applied constantly
301 to 35.5%-50.0% of the monitored breeding population yearly ($p=0.05$) (Table 1). The
302 probability of detecting a swIAV in ILI pigs was not different in vaccinated and non-
303 vaccinated herds ($p=0.09$) (Table 2).

304 Antipyretic agents were given to sick pigs as mass medication in 26% of herds on average
305 (from 18% to 32% depending on the year), while antibiotic treatment was applied in 19% of
306 cases on average (from 15% to 27%). These drug prescriptions remained stable over time
307 ($p=0.07$ and $p=0.1$, respectively) (Table 1).

308 Both growing pigs and breeders exhibited ILI, but growing pigs were more frequently
309 statistically associated with swIAV detection than gilts and sows (52% versus 26%) ($p<0.001$)
310 (Table 2). The frequency of swIAV-positive cases was higher when ILI-affected animals were
311 piglets under 10 weeks of age, as compared to older animals (51.6% [≤ 6 weeks] and 56.1%
312 [$6;9$ weeks] versus 46.8% [>12 weeks], $p=0.001$). Animals with hyperthermia (rectal
313 temperature $> 40.5^{\circ}\text{C}$) were more frequently detected swIAV-positive than animals without
314 fever (52% versus 43.8%) ($p=0.02$) (Table 2).

315 **Clinical signs and epidemiological patterns associated with swIAV infections (univariate** 316 **analyses)**

317 For most of the confirmed swIAV-positive cases, veterinarians indicated that pigs exhibited
318 clinical signs of moderate intensity, i.e., lasting no more than 2–3 days at the individual level
319 and without mortality. However, up to approx. 30% of swIAV-positive cases were reported
320 yearly to have shown severe clinical signs, i.e., lasting longer than usual and/or with some
321 associated mortality (Table 1). In 2011 and 2012, the annual frequency of outbreaks of high-
322 clinical intensity were lower as in following years, probably due to a lack of awareness among

323 veterinarians towards this variable. However, since 2013, this frequency appeared stable from
324 one year to the next, ranging from 17.9% to 29.5% ($p=0.63$) (Table 1). The proportion of
325 cases with high intensity clinical signs was higher from December to March than from April
326 to November (24.5% versus 18.9%, $p=0.03$) (Table 3). High intensity ILI affected all animal
327 types, regardless of their physiological stage or age. However, when considering the different
328 age groups within growing pigs, it appeared that piglets infected before or at 6 weeks of age
329 were at a lower risk of exhibiting clinical signs of high intensity than older pigs ($p=0.006$)
330 (Table 3). No significant association was found between either the intensity of clinical signs
331 and the swIAV subtype ($p=0.3$), or between the intensity of clinical signs and the vaccination
332 program applied in the herd ($p=0.11$) (Table 3).

333 Pigs were predominantly affected by the classical epidemiological pattern of influenza.
334 However, recurrent disease, i.e., an enzootic form of the infection at the herd level, was
335 regularly reported and accounted for 41.1% (345/839) of the swIAV-confirmed cases since
336 2011, without marked differences between years ($p=0.2$) (Table 1). Among swIAV-positive
337 cases, recurrent swIAV infection was more frequently reported in herds located in
338 administrative regions with the largest pig populations ($p<0.001$), as well as in herds with
339 breeders ($p<0.001$) (Table 4). Moreover, the proportion of recurrent infections was
340 significantly higher in vaccinated herds than in non-vaccinated ones, 52.0% versus 34.4%
341 ($p<0.001$) (Table 4). Finally, recurrent influenza was more frequently reported than the
342 classical pattern in batches of post-weaning piglets. Thus, the median ages at infection were 7
343 and 12 weeks of age in both populations, respectively (Fig. 5). The frequency of youngest (\leq
344 6 week-old) swIAV-positive pigs affected by recurrent infection reached 64.2%, in contrast to
345 only 19.7% within the oldest pigs (>12 weeks old) ($p<0.001$) (Table 4). The epidemiological
346 pattern was not significantly related to either the intensity of the clinical signs ($p=0.9$), or to
347 the swIAV subtype ($p=0.9$) (Table 4).

348 **Multivariate assessment of variables related to swIAV herd positivity, clinical signs and**
349 **associated epidemiological patterns**

350 The investigation of potential statistical associations between herd positivity to swIAV and
351 the characteristics of the pig population size, the presence or absence of breeders on site, the
352 application or not of a vaccination program, the age of growing pigs (<10 and \geq 10 weeks of
353 age) and rectal temperature (below or above \geq 40.5°C) did not show any significant links
354 ($p=0.42$, 0.31, 0.25, 0.22 and 0.15, respectively) between these variables. No significant
355 association was found between the intensity of clinical signs and the vaccination policy
356 ($p=0.08$), or between the intensity of clinical signs and pig age ($p=0.24$). In contrast, the
357 multivariate assessment confirmed the associations between the recurrent epidemiological
358 pattern of swIAV infection and both the vaccination of breeders and the age of growing pigs
359 (Table 5).

360

361 **Discussion**

362 During its first seven years of activity, the RESAVIP network investigated a substantial
363 number of ILI pigs located throughout mainland France. The design of the surveillance
364 system enabled swIAV detection in around 50% of investigated farms by testing only three
365 pigs per affected batch, despite many other pathogens that may be responsible for respiratory
366 disease. This rate was similar to or even higher than the rates obtained in other surveillance
367 programs (Baudon et al., 2017; Fablet et al., 2012; Henritzi et al., 2016; Loeffen et al., 1999;
368 Simon et al., 2014), with our program having limited analytical costs and avoiding the need to
369 pool samples.

370 The substantial number of ILI investigations consolidated the knowledge on swIAV
371 circulation in France, although regions with small pig population sizes provided few samples.
372 Factors related to the swine sector organization would interfere with ILI monitoring in regions
373 with the smallest pig populations, such as a restricted number of veterinarians each covering
374 large territories and/or less sensitivity of breeders to clinical signs. Nevertheless, infected
375 farms were identified throughout the country, allowing consideration that the network
376 provided a good picture of the dynamics of swIAVs in circulation in France. A forthcoming
377 serological survey as the one conducted before the 2009 pandemic (Hervé et al., 2011) could
378 consolidate the passive surveillance, especially in areas with smallest pig populations.

379 SwIAVs from the four European enzootic lineages were identified, in descending order of
380 proportions, H1_{av}N1, H1_{hu}N2, H1N1pdm, and H3N2. H1_{av}N2 reassortants and several virus
381 mixtures were also found. These results are in accordance with and complementary to those
382 obtained by the NRL through other sampling sources, e.g., requests for diagnostic analyses or
383 epidemiological studies, with the exception of H1_{hu}N1 reassortants that were isolated twice in
384 2011 and 2012 (Simon et al., 2013). While H1_{av}N1 strains were detected all around the
385 country irrespective of pig population size, as in many other European countries (Watson et
386 al., 2015), H1_{hu}N2 and its antigenic variant H1_{hu}N2v were detected only in the north-western
387 part of France for many years, until H1_{hu}N2 detection in the North from 2016. Although the
388 proportion of H1_{hu}N2v declined in recent years, this variant could still be circulating, but
389 ongoing monitoring will give more information on its survival. In Italy, a similar variant
390 seems to have disappeared after it was observed for several years (Moreno et al., 2013). Over
391 the study period, it appeared that H1N1pdm viruses did not establish in the north-western part
392 of the country, the area with the largest pig population size, possibly due to competition with
393 other previous enzootic viruses that were very highly prevalent in this region (Chastagner et
394 al., 2018). By contrast to other countries, reassortants exhibiting H1pdm or N1pdm genes

395 were not detected in France until March 2018 (Krog et al., 2017). However, virus mixtures
396 containing H1N1pdm genes were observed, demonstrating co-infection events; thus, new
397 reassortants may be expected in the future. After the disappearance of H3N2 in the North-
398 west at the end of the 1990's, this swIAV was detected in herds located in the North in 2012
399 (Hervé et al., 2012), and RESAVIP confirmed its presence close to the Belgian border,
400 probably in connection with the import of infected animals. Thus, the surveillance network
401 has highlighted increasing swIAV diversity in this area in the last few years. Co-circulations
402 increase the probability of co-infections and reassortment events. RESAVIP identified
403 H1_{av}N2 reassortants in several regions, mostly in the western part of the country for years, but
404 also in the North in 2016 (Bonin et al., 2016). More surprising was the identification of
405 H1_{av}N2 viruses in the south-western part of France, where enzootic HxN2 viruses had never
406 been detected before, leading us to hypothesize that these reassortants were introduced *in toto*
407 by animals coming from other French regions or European countries (Bonin et al., 2016;
408 Watson et al., 2015). To date, the H1_{av}N2 viruses do not appear to be novel enzootic viruses
409 that would circulate within the French pig population, but this could occur, as previously
410 observed in Denmark and the United Kingdom for example (Simon et al., 2014). Therefore,
411 their potential propagation will need to be monitored closely.

412 Whereas RESAVIP enabled the identification of 669 swIAVs out of 887 swIAV-positive
413 cases, 25% (167/669) remained partially subtyped or non-subtyped, as observed elsewhere
414 (Haach et al., 2019; Simon et al., 2014). This was mainly due to the higher sensitivity of the
415 commercial M gene RT-qPCRs as compared to the in-house HA and NA subtyping methods,
416 the former using TaqMan[®] MGB probes (Bonin et al., 2018; Pol et al., 2011).

417 The data accumulated during the seven years of monitoring did not reveal any impact of
418 month or season on the proportion of swIAV-positive cases, regardless of the virus subtype.
419 This was also observed in closed intensive herds in Canada (Poljak et al., 2014). To date, a

420 cyclical pattern of swIAV circulation in pigs has only been demonstrated on breed-to-wean
421 farms in the United States, through a 5-year study (Chamba Pardo et al., 2017). Two peaks of
422 swIAV-positive cases, whether associated with clinical disease or not, were distinguished in
423 each early winter (December) and late spring (May). They were hypothesized to relate to
424 variations in air humidity and temperature, in line with IAV survival in the environment and
425 patterns of epidemics in humans (Rambaut et al., 2008). In intensive herds in France, the fact
426 that swIAV infections occur all year round could be explained by stable environmental
427 conditions in closed buildings, whereas seasonal occurrence of potential asymptomatic
428 infections cannot be ruled out; however, these types of silent infections were not investigated
429 in the RESAVIP passive surveillance program. Nevertheless, when discriminating swIAV
430 infections depending on the virus lineage, it appeared that the proportion of detected
431 H1N1pdm viruses significantly increased in the winter months, especially over the most
432 recent years and during seasonal influenza epidemics when H1N1pdm was the dominant IAV
433 infecting humans, as in 2016 for example. Thus, *de novo* transmissions from humans to pigs
434 probably occurred during these periods, alongside intra-species H1N1pdm circulation since
435 2010 (Chastagner et al., 2018; Watson et al., 2015). This is consistent with phylogenetic
436 analyses that estimated many inter-species transmission events since the pandemic
437 (Chastagner et al., 2018; Nelson et al., 2012). The concurrent decrease in detected H1_{av}N1
438 viruses in the same months could be related to potential competition between both H1N1
439 subtypes in winter, but not outside the human seasonal epidemic when infection pressure is
440 lower. However, further investigations and continued monitoring would be necessary to
441 confirm this hypothesis, as well as to understand the higher proportion of H1_{hu}N2 in winter.

442 Three quarters of visited farms where ILI pigs were sampled were farrow-to-finish herds,
443 while this production type accounts for approximately 36.5% of pig herds only, according to
444 the national swine identification database (BDporc). However, no statistical link was found

445 between swIAV detection and the presence of breeders in the herd. Interestingly, recurrent
446 infections affected preferentially piglets in nursery, consistent with previous longitudinal
447 studies (Rose et al., 2013). From the data obtained in this study, we also found that the
448 recurrent swIAV infection form was related to the vaccination of breeders in herds. This could
449 be in line with experimental and field studies that pointed out a potential adverse effect of
450 maternally-derived antibodies (MDAs) initially thought to protect the piglets at least at the
451 clinical level (Deblanc et al., 2018; Loeffen et al., 2003). In fact, whereas animals infected in
452 the presence of passive immunity were shown to be less infectious than piglets without MDA,
453 they still shed infectious viral particles with an extended spread of the disease at the batch
454 level, a phenomenon that would contribute to the persistence of the pathogen within the farm
455 (Cador et al., 2016; Rose et al., 2013). However, from the surveillance data, no causal
456 relationships between recurrent infections and vaccination can be ascertained, as it cannot be
457 excluded that vaccination is motivated by recurrent ILI.

458 While no statistical link was observed between sow vaccination and the intensity level of
459 respiratory outbreaks, the monitoring showed that piglets under 6 weeks old became infected
460 with a lower risk of developing severe clinical signs, as compared to older animals. As these
461 young animals are also those with potentially the highest MDA levels, they might have been
462 partially protected, as demonstrated experimentally (Deblanc et al., 2018; Loeffen et al.,
463 2003).

464 No statistical relationship between the virus subtype and the intensity of the clinical signs was
465 found, whereas field observations and experimental studies have suggested that the H3N2 and
466 H1N2 viruses were more pathogenic than viruses of the H1N1 subtype (Deblanc et al., 2012;
467 Krog et al., 2017; Zhou et al., 1999). No statistical link was established between the virus
468 subtype and the epidemiological pattern of the disease. A longitudinal survey suggested that
469 recurrent infections could favour concomitant swIAV infections (Rose et al., 2013), but here

470 the virus mixtures could not be related to this epidemiological form, given the surveillance
471 design with independent event-based visits.

472 Although restricted to the studied pig population, the cumulative data from this surveillance
473 network provided strong information about the diversity and dynamics of swIAVs in France.
474 The significant statistical associations found in this study encourage continued monitoring of
475 swIAV infections in the framework of RESAVIP.

476

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487

488 **List of abbreviations**

489 swIAV = swine influenza A virus, HA = hemagglutinin, NA = neuraminidase, H1_{av}N1 =
490 European avian-like swine H1N1, H1N1_{pdm} = pandemic-like swine H1N1, H1_{hu}N2 =
491 European human-like reassortant swine H1N2, H3N2 = European human-like reassortant
492 swine H3N2, ILI = influenza-like illness, RESAVIP = French national surveillance network

493 for swIAVs, SAD = specific accompanying datasheet, GEEs = generalized estimating
494 equations

495 **Competing interests**

496 The authors declare that they have no competing interests.

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625

626

627 **Figure captions**

628 **Figure 1: Monthly distribution of numbers of swIAV-negative (in black) and swIAV-**
629 **positive (in red) pig herds investigated in France by RESAVIP from April 2011 to**
630 **March 2018.**

631 **Figure 2: Geographical distribution of herds visited in mainland France by RESAVIP**
632 **from April 2011 to March 2018 at the administrative region level.**

633 Each circle shows the distribution of IAV-negative (in black) and IAV-positive (in red) pig
634 herds. The four different circle sizes represent the numbers of visited herds within the study
635 period: [1; 10[, [10; 100[, [100; 500[and ≥ 500 from the smallest to the largest, respectively.
636 The administrative *départements* are colored in grey gradient according to the size of their pig
637 population, based on the data provided by the National Agricultural Census in 2010 divided
638 into 3 classes (30).

639 **Figure 3: Distribution of annual proportions of the different swIAV lineages identified**
640 **by RESAVIP from April 2011 to March 2018.**

641 Pink bars represent the proportion of H1_{av}N1 viruses, red bars are H1N1pdm, light blue bars
642 are H1_{hu}N2, dark blue bars are H1_{hu}N2 variant, green bars are H3N2, purple bars are H1_{av}N2,
643 and brown bars are virus mixtures. 2011 and 2018 are incomplete years, with 9 and 3 months
644 only, respectively.

645 **Figure 4: Relative frequencies of the different swIAV lineages identified by RESAVIP in**
646 **each administrative region of mainland France from April 2011 to March 2018.**

647 Each circle represents the distribution of identified swIAVs according to their genetic
648 lineages: H1_{av}N1 are colored in pink, H1N1pdm in red, H1_{hu}N2 in light blue, H1_{hu}N2 variant
649 in dark blue, H3N2 in green, H1_{av}N2 in purple, and virus mixtures in brown. The four

650 different circle sizes represent the numbers of swIAVs identified within the study period: [1;
651 10[, [10; 50[, [50; 100[and [100; 300[from the smallest to the largest, respectively. The
652 administrative *départements* are colored in grey gradient according to the size of their pig
653 population, based on the data provided by the National Agricultural Census in 2010 divided
654 into 3 classes (30).

655 **Figure 5: Distribution of growing pig ages in swIAV-positive herds depending on the**
656 **epidemiological pattern associated with the influenza-like illness at sampling, i.e.,**
657 **classical (light yellow bars) or recurrent (yellow bars).**

658

659 **Appendix**

660 **Sample Accompanying Datasheet (SAD)**

661 **Tables**

662 **Table 1: Annual distribution of tested cases according to swIAV detection, mass medication,**
 663 **intensity of clinical signs in swIAV-positive cases, and epidemiological pattern in swIAV-positive**
 664 **cases (numbers of tested cases and frequencies in brackets).**

Year	swIAV detection by M-gene RT-qPCR		Mass medication (swIAV-positive and negative cases)			Intensity of clinical signs (swIAV-positive cases)		Epidemiological pattern (swIAV- positive cases)	
	Positive	Negative	Vaccination	Antipyretic treatment	Antibiotic treatment	Moderate	High	Classical	Recurrent
2011^a	68 (64.8)	37 (35.2)	nd ^b	nd	nd	53 (85.5)	9 (14.5)	50 (74.6)	17 (25.4)
2012	133 (53.6)	115 (46.4)	nd	nd	nd	109 (94.0)	7 (6.0)	74 (57.4)	55 (42.6)
2013	136 (49.3)	140 (50.7)	108 (44.4)	63 (27.9)	41 (22.7)	93 (76.9)	28 (23.1)	72 (57.1)	54 (42.9)
2014	128 (47.2)	143 (52.8)	108 (40.6)	74 (28.8)	37 (18.5)	94 (78.3)	26 (21.7)	70 (57.4)	52 (42.6)
2015	139 (48.6)	147 (51.4)	99 (35.5)	89 (32.0)	38 (17.0)	103 (76.3)	32 (23.7)	82 (61.7)	51 (38.3)
2016	138 (44.7)	171 (55.3)	118 (38.7)	67 (22.9)	36 (15.1)	93 (70.5)	39 (29.5)	67 (51.9)	62 (48.1)
2017	102 (41.8)	142 (58.2)	111 (46.8)	58 (25.0)	29 (15.3)	69 (75.0)	23 (25.0)	55 (59.1)	38 (40.9)
2018^c	43 (50.0)	43 (50.0)	41 (50.0)	15 (18.3)	18 (27.3)	32 (82.1)	7 (17.9)	24 (60.0)	16 (40.0)
<i>p</i> -value ^d	0.20		0.05	0.07	0.1	<0.001		0.20	

665

666 ^a9 months

667 ^bnd = not determined

668 ^c3 months

669 ^dPearson's Chi-squared test

670

671 **Table 2: Distribution of swIAV-positive and negative cases according to seven explanatory**
672 **variables: univariate analysis**

Variable	Total number of available data	Number of swIAV-positive cases (%)	Number of swIAV-negative cases (%)	<i>p</i> -value ^a
Season				
from April to November	1142	565 (49.5)	577 (50.5)	0.53
from December to March	683	322 (47.1)	361 (52.9)	
Pig population size				
small <150.000	96	44 (45.8)	52 (54.2)	0.01
medium [150.000 – 500.000[309	130 (42.1)	179 (57.9)	
large ≥500.000	1420	713 (50.2)	707 (49.8)	
Type of production farm				
with breeders ^b	1491	734 (49.2)	757 (50.8)	0.27
without breeders ^c	330	152 (46.1)	178 (53.9)	
Vaccination program^d				
No vaccination	829	379 (45.7)	450 (54.3)	0.09
Vaccination	719	357 (49.7)	362 (50.3)	
Animals				
breeders	252	65 (25.8)	187 (74.2)	<0.001
growing pigs	1530	798 (52.2)	732 (47.8)	
Age of growing pigs				
≤6 weeks	382	197 (51.6)	185 (48.4)	0.001
]6-9] weeks	399	224 (56.1)	175 (43.9)	
]9-12] weeks	119	66 (55.4)	53 (44.5)	
>12 weeks	539	252 (46.8)	287 (53.2)	
Rectal temperature^d				
<40.5°C	735	322 (43.8)	413 (56.2)	0.02
≥40.5°C	593	308 (51.9)	285 (48.1)	

673

674 ^aGEE model

675 ^bfarrowing-to-weaning+farrowing-to-finishing+farrowing-post weaning

676 ^cfinishing+post weaning-finishing+post weaning

677 ^ddata only available from January 2013 to March 2018

678

679 **Table 3: Distribution of moderate or high intensity swIAV-positive cases according to four**
 680 **explanatory variables: univariate analysis**

Variable	Total number of available data	Number of respiratory outbreaks of moderate intensity (%)	Number of respiratory outbreaks of high intensity (%)	<i>p</i> -value
Vaccination program^a				
no vaccination	356	266 (74.7)	90 (25.3)	0.11 ^b
vaccination	330	263 (79.7)	67 (20.3)	
Season				
from April to November	523	424 (81.1)	99 (18.9)	0.03 ^b
from December to March	294	222 (75.5)	72 (24.5)	
Age of growing pigs				
≤6 weeks	177	147 (83.1)	30 (16.9)	0.006 ^b
]6-9] weeks	208	167 (80.3)	41 (19.7)	
]9-12] weeks	64	47 (73.4)	17 (26.6)	
>12 weeks	238	188 (79.0)	50 (21.0)	
Virus subtype				
H1 _{av} N1	425	332	93	0.3 ^c
H1N1pdm	24	21	3	
H1 _{hu} N2	96	77	19	
H1 _{hu} N2v	47	30	17	
H1 _{av} N2	9	7	2	
H3N2	4	4	0	
virus mixtures	16	13	3	

681

682 ^adata only available from January 2013 to March 2018

683 ^bGEE model

684 ^cFisher's Exact test

685

686 **Table 4: Distribution of swIAV-positive cases of classical or recurrent epidemiological patterns**
 687 **according to six explanatory variables: univariate analysis**

Variable	Total number of available data	Number of classical epidemiological patterns (%)	Number of recurrent epidemiological patterns (%)	<i>p</i> -value
Pig population size				
small <150.000	42	29 (69.0)	13 (31.0)	<0.001 ^a
medium [150.000 – 500.000[120	81 (67.5)	39 (32.5)	
large ≥500.000	677	384 (56.7)	293 (43.3)	
Type of production farm				
with breeders ^b	696	394 (56.6)	302 (43.4)	<0.001 ^a
without breeders ^c	142	100 (70.4)	42 (29.6)	
Vaccination program^d				
No vaccination	355	233 (65.6)	122 (34.4)	<0.001 ^a
vaccination	342	164 (48.0)	178 (52.0)	
Age of growing pigs				
≤6 weeks	187	67 (35.8)	120 (64.2)	<0.001 ^a
]6-9] weeks	211	95 (45.0)	116 (55.0)	
]9-12] weeks	64	40 (62.5)	24 (37.5)	
>12 weeks	244	198 (81.1)	46 (18.9)	
Intensity of clinical signs				
moderate	626	372 (59.4)	254 (40.6)	0.9 ^a
high	163	96 (58.9)	67 (41.1)	
Virus subtypes				
H1 _{av} N1	437	269	168	0.9 ^e
H1N1pdm	24	17	7	
H1 _{hu} N2	97	58	39	
H1 _{hu} N2v	48	32	16	
H1 _{av} N2	8	4	4	
H3N2	4	3	1	
virus mixtures	16	9	7	

688

689 ^aGEE model

690 ^bfarrowing-to-weaning+farrowing-to-finishing+farrowing-post weaning

691 ^cfinishing+post weaning-finishing+post weaning

692 ^ddata only available from January 2013 to March 2018

693 ^eFisher's Exact test

694

695

696 **Table 5: Final multivariate model (GEE regression model) of explanatory variables related to**
697 **the epidemiological pattern (recurrent versus classical) in swIAV-positive cases (887 cases, 2011-**
698 **2018) - Estimate, Standard Error and OR (adjusted Odds Ratio) with 95% confidence interval**
699 **(CI)**

Variable	Estimate	Standard Error	OR (95% CI)	<i>p</i> -value ^a
Pig population size				
large versus small	0.45	0.56	1.57 (0.53, 4.69)	0.35
medium versus small	0.42	0.67	1.52 (0.40, 5.68)	
large versus medium	0.03	0.16	1.03 (0.76, 1.41)	
Type of production farm				
with breeders versus without	0.09	0.10	1.10 (0.90, 1.34)	0.45
Vaccination program^b				
Vaccinated versus non vaccinated	0.67	0.18	1.96 (1.37, 2.80)	0.02
Age of growing pigs				
<10 weeks versus ≥10 weeks	1.63	0.21	5.11 (3.36, 7.76)	0.02

700

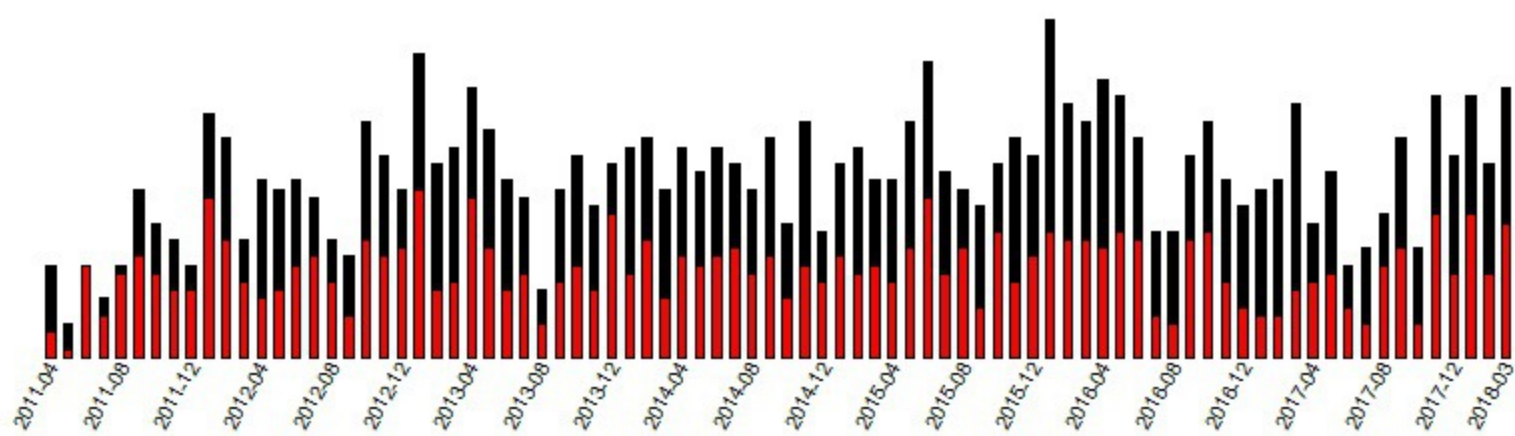
701 ^aGEE model

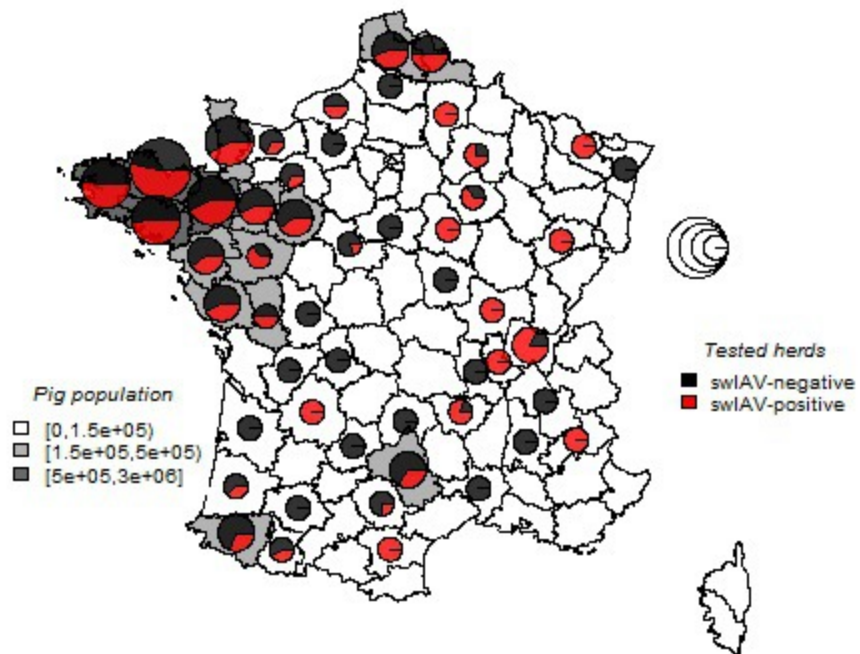
702 ^bdata only available from January 2013 to March 2018

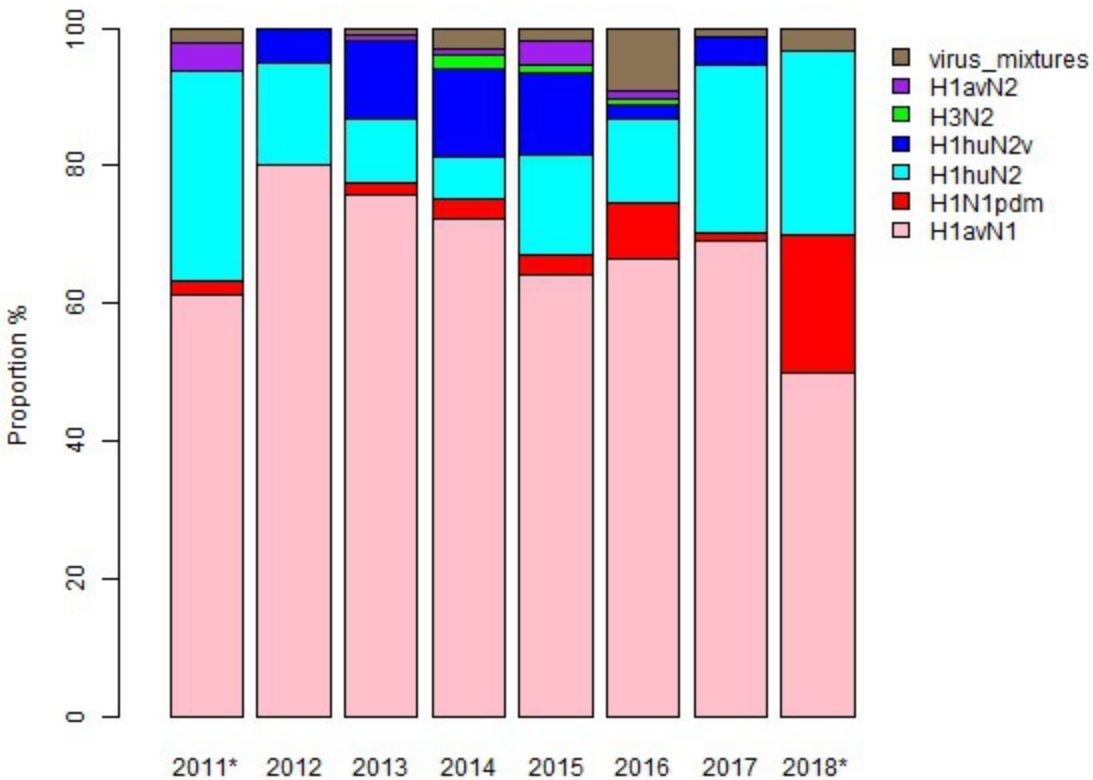
Pig herds number

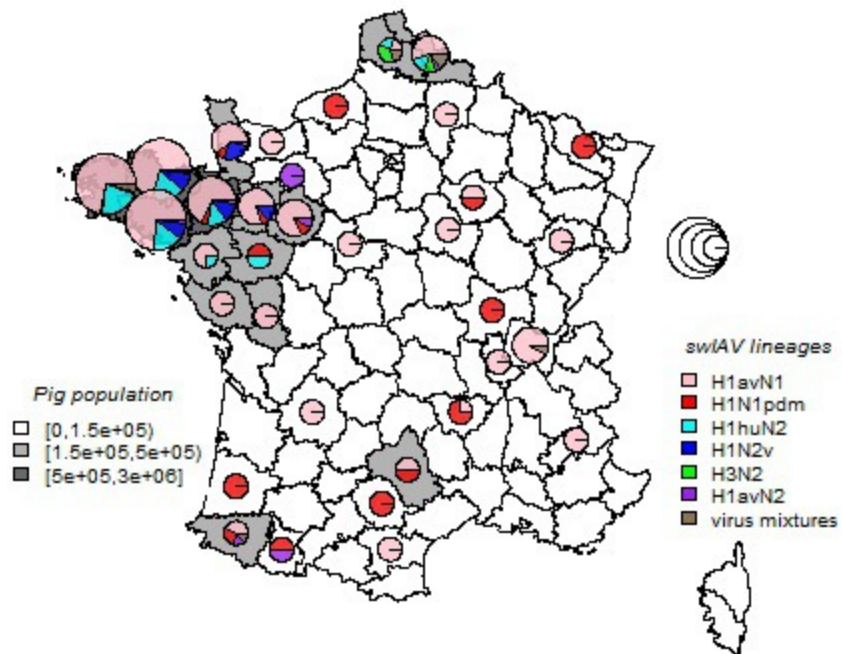
40
30
20
10
0

2011-04 2011-08 2011-12 2012-04 2012-08 2012-12 2013-04 2013-08 2013-12 2014-04 2014-08 2014-12 2015-04 2015-08 2015-12 2016-04 2016-08 2016-12 2017-04 2017-08 2017-12 2018-03









Number of swIAV-positive herds

