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

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ABSTRACT

Swine influenza A viruses (swIAVs) cause acute respiratory syndromes in pigs and may also infect humans. Following the 2009 pandemic, a network was established in France to reinforce swIAV monitoring. This study reports virological and epidemiological data accumulated through passive surveillance conducted during 1,825 herd visits from 2011 to 2018. Among them, 887 (48.6 %) tested swIAV-positive. The proportion of positive cases remained stable year-on-year and year-round. The European avian-like swine H1N1 ($H1_{av}N1$) virus was the most frequently identified (69.6 %), and was widespread across the country. The European human-like reassortant swine H1N2 ($H1_{hu}N2$) virus accounted for 22.1 % and was only identified in the north-western quarter and recently in the far north. The 2009 pandemic H1N1 (H1N1pdm) virus (3.6 %) was detected throughout the country, without settling in areas of higher pig densities. Its proportion increased in winter, during the seasonal epidemics in humans. The European human-like reassortant swine H3N2 as well as $H1_{av}N2$ viruses were identified sporadically. In up to 30 % of swIAV-positive cases, pigs exhibited clinical signs of high intensity, regardless of the viral subtype and vaccination program. The recurrent pattern of the disease, i.e., an endemic infection at the herd level, was reported in 41% of cases and mainly affected post-weaning piglets (OR = 5.11 [3.36–7.76]). Interestingly, the study also revealed a significant association between the recurrent pattern and sow vaccination (OR = 1.96 [1.37–2.80]). Although restricted to the studied pig population, these results bring new knowledge about swIAV dynamics and infection patterns in pig herds in France.

Abbreviations: swIAV, swine influenza A virus; HA, hemagglutinin; NA, neuraminidase; $H1_{av}N1$, European avian-like swine H1N1; H1N1pdm, pandemic-like swine H1N1; $H1_{hu}N2$, European human-like reassortant swine H1N2; H3N2, European human-like reassortant swine H3N2; ILI, influenza-like illness; RESAVIP, French national surveillance network for swIAVs; SAD, specific accompanying datasheet; GEEs, generalized estimating equations

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1. Introduction

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

SwIAVs may also be transmitted to humans where they can cause influenza and have sometimes led to epidemics. In 2009, a novel H1N1 virus of swine origin emerged in humans and was responsible for the first influenza pandemic of the 21st century, before becoming seasonal in humans as well as enzootic in pig populations worldwide after reverse zoonosis (Adlhoch et al., 2014; Simon et al., 2014). Following the 2009 pandemic, research teams, expert laboratories, and international organizations (WHO/OIE/FAO) launched a request to reinforce swIAV surveillance in order to better understand the emergence of new viruses (Vincent et al., 2014). In Europe, the European Surveillance Network for Influenza in Pigs (ESNIP) showed that viruses from three subtypes (HxNy) and four genetic lineages, defined by the nature and origin of the genes encoding the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins, were circulating enzootically in the European pig population (Simon et al., 2014). These predominant lineages were the European avian-like swine H1N1 ($H1_{av}N1$), the pandemic-like swine H1N1 (H1N1pdm), the European human-like reassortant swine H1N2 ($H1_{hu}N2$), and the European human-like reassortant swine H3N2 (H3N2). They were detected with variable frequencies depending on the geographical area. Their co-circulation induced reassortant viruses through genomic reassortment, i.e., exchange of one or several genomic segments between different viruses through coinfection events, among which some became enzootic in certain countries (Watson et al., 2015). Moreover, mutations in the HA and/or NA genes can lead to the production of variants that are antigenically distant from their parental viruses, contributing to the emergence of new swIAVs. As an example, a novel variant called $H1_{hu}N2_{\Delta 146\text{-}147}$ ($H1_{hu}N2v$) was detected in France in 2012 following genetic and antigenic drift in the parental $H1_{hu}N2$ lineage (Bonin et al., 2018).

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

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

2. Materials & methods

2.1. The RESAVIP network

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

2.2. Case definition, biological samples and epidemiological data collection

Biological samples were taken from pigs exhibiting ILI by a volunteer veterinarian, either after a call from a farmer to report an outbreak, or during a previously planned herd visit. In this study, all visits were considered to be independent of each other, even if some herds could have been visited more than once during the 7-year monitoring. In each affected herd, nasal swabs (Sigma Virocult®, Kitvia, Labarthe-Inard, France) were collected from three growing pigs of the same age, or three breeders, that were selected based on their rectal temperature (above 40.5 °C preferentially). This constituted a suspected case. The case was considered to be swIAV-positive if at least one of the three samples tested positive in the first-line laboratory detection test (see below).

In addition to biological samples, the veterinarian collected outbreak-related information on a specific accompanying datasheet (SAD), asking for information about the sampling context and date, the geographic location of the farm at the French administrative region level (administrative area of 6,130 km^2 on average), the herd type, the vaccination program, the age and/or physiological stage of the sampled animals, the individual rectal temperatures, the medication applied, the ILI intensity, and the epidemiological pattern of the outbreak (see details in Appendix 1 (in the Supplementary material)).

2.3. swIAV detection and subtyping

Nasal swab supernatants were submitted to accredited local public veterinary laboratories for swIAV M gene real-time RT-PCR (RT-qPCR) using commercial kits previously validated by the NRL, LSI VetMAX Swine Influenza A-A/H1N1/2009 included (LSI-Life Technologies, Lissieu, France) or Adiavet SIV Real Time (ADIAGENE-BioX Diagnostics, Ploufragan, France) (Pol et al., 2011). The analytical specificity and sensitivity of both tests were 100 % each. All M gene-positive samples (Cq values < 45), as well as corresponding viral RNA extracts, were sent to the NRL for subsequent subtyping using RT-qPCR assays that specifically amplify and discriminate the HA and NA genes from the different lineages of swIAVs known to be enzootic in European pig herds ($H1_{av}N1$, $H1_{hu}N2$, H3N2, and H1N1pdm). Briefly, three in-house RT-qPCR assays allowing the amplification of the $H1_{av}$, $H1_{hu}$ and H3 genes, respectively, and one duplex RT-qPCR for the detection of the N1 and N2 genes were applied, as previously described (Bonin et al., 2018). The N1 RT-qPCR amplifies NA genes from both the $H1_{av}N1$ and the H1N1pdm lineages, whereas the N2 RT-qPCR amplifies NA genes from both the $H1_{hu}N2$ and the H3N2 lineages. When an $H1_{hu}Ny$ virus was detected, another RT-qPCR was run that specifically targets an $H1_{hu}$ variant gene ($H1_{hu}v$), which was first detected in France in 2012 (Bonin et al., 2018). RT-qPCRs for the amplification of the H1pdm and N1pdm genes were carried out using commercial kits previously validated by the NRL, Adiavet A/H1N1 2009 Real Time (ADIAGENE-BioX Diagnostics, Ploufragan, France) or LSI VetMAX Swine Influenza A/H1N1/2009-H1 Detection for H1pdm, and LSI VetMAX Swine Influenza A/H1N1/2009-N1 Detection (LSI-Life Technologies, Lissieu, France) for N1pdm (Pol et al., 2011). Due to the lower sensitivity of in-house subtyping methods as compared to the swIAV detection method, the HA- and NA-gene RT-qPCRs were only run on viral RNA extracts exhibiting Cq values < 35 in M gene RT-qPCR (Bonin et al., 2018). When HA and/or NA gene identification was unsuccessful, but the initial M gene RT-qPCR Cq value was < 30, the virus detected in nasal swab supernatant was propagated on MDCK cells (WHO, 2002), and further subtyping analyses were performed on the isolated strain.

2.4. Epidemiological data analyses

The epidata reported on SADs were collected by RESAVIP regional coordinators and entered into regional databases that were integrated every quarter into a national database managed by the national coordinator. The temporal and spatial distributions of the different swIAV lineages were collated from the collected epidata and results of lab analyses. Three outcomes were also investigated in this study: (1) case positivity to swIAVs, (2) severity of clinical signs (moderate or high), and (3) epidemiological patterns of swIAV infection (classical or recurrent). The classic form is defined by a sporadic acute infection and the recurrent form is related to an endemic pattern of infection at the herd level, affecting each successive batch of pigs at a given physiological stage.

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

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

3. Results

3.1. Temporal and spatial distribution of the pig farms included in RESAVIP

From April 2011 to March 2018, the RESAVIP network investigated 1,825 ILI cases (261 farm visits per year on average, min 244, max 309) (Table 1). The completeness of the data collected in SADs varied from 78.7 to 100 %, depending on the variables. In 80 % of cases, the herd was included in RESAVIP after the farmer called the veterinarian to report a respiratory outbreak. The remaining 20 % of veterinary visits were already scheduled. Importantly, nearly 55 % of the farm visits (739/1,351) took place less than 72 h after the first ILI clinical signs were observed by the farmer. However, ILI started 3 to 7 days before the sampling date in almost 34 % of cases (458/1,351), and more than one week prior to sampling in just over 11 % of the visits (154/1,351). The frequency of swIAV-positive herds decreased as the time between the onset of clinical signs and sampling increased (51.8 %, 45.0 % and 32.5% when sampling took place less than 3 days, between 3 and 7 days, or more than 7 days after ILI occurrence, respectively, $p < 0.001$), but remained relatively stable over the years, ranging from 41.8 to 53.6 % ($p = 0.20$) (Table 1). The swIAV genome was detected in

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

	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)	
Year	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)
p-value[d]	0.20		0.05	0.07	0.1	< 0.001		0.20	

[a] 9 months.
[b] nd = not determined.
[c] 3 months.
[d] Pearson's Chi-squared test.

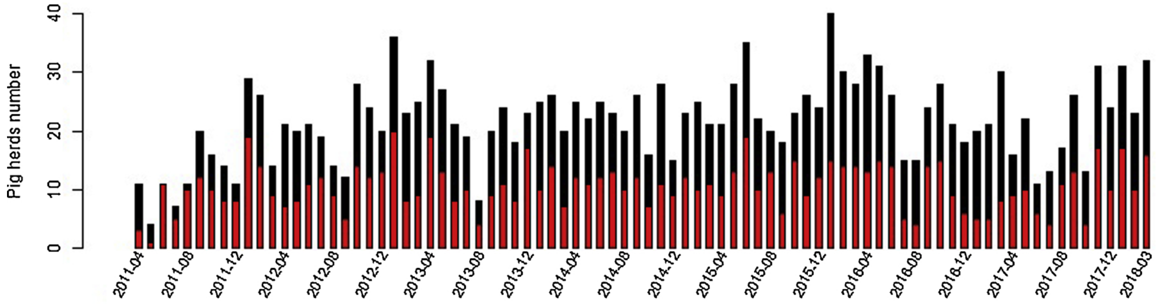

Fig. 1. Monthly distribution of numbers of swIAV-negative (in black) and swIAV-positive (in red) pig herds investigated in France by RESAVIP from April 2011 to March 2018. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the three collected nasal swabs in three quarters of the positive cases (data not shown). Positive and negative herds were reported every month during the study period (Fig. 1), showing that swIAV infections as well as ILI due to other respiratory pathogens occurred throughout the year, with similar monthly distributions ($p = 0.43$). Also, the proportion of herds that were detected swIAV positive in winter, i.e., from December to March when the seasonal epidemic occurs in humans, was not different to that of positive herds sampled from April to November: 47.1 % versus 49.5 % ($p = 0.53$) (Table 2).

The visited herds were located in 51 administrative regions out of 96 (53 %) in mainland France, across the country (Fig. 2). At least one swIAV-positive herd was detected in 35 out of these 51 administrative regions across the territory (Fig. 2). The proportion of swIAV-positive herds was significantly higher in administrative regions with the largest pig population size than in those with a medium-sized population (50.2 % versus 42.1 %, $p = 0.01$) (Table 2).

3.2. Relative frequencies and temporal and spatial distribution of virus subtypes

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

Other viruses of the H1N1 subtype belonged to the H1N1pdm lineage. Since RESAVIP was set up, this lineage has been identified each year except in 2012, accounting for 3.6 % (24/669) of the subtyped viruses, with annual proportions varying from 1.3 % (1/74) in 2017 to

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

Variable	Total number of available data	Number of swIAV-positive cases (%)	Number of swIAV-negative cases (%)	*p*-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				0.01
small < 150.000	96	44 (45.8)	52 (54.2)	
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)	

[a] GEE model.
[b] farrowing-to-weaning + farrowing-to-finishing + farrowing-post weaning.
[c] finishing + post weaning-finishing + post weaning.
[d] data only available from January 2013 to March 2018.

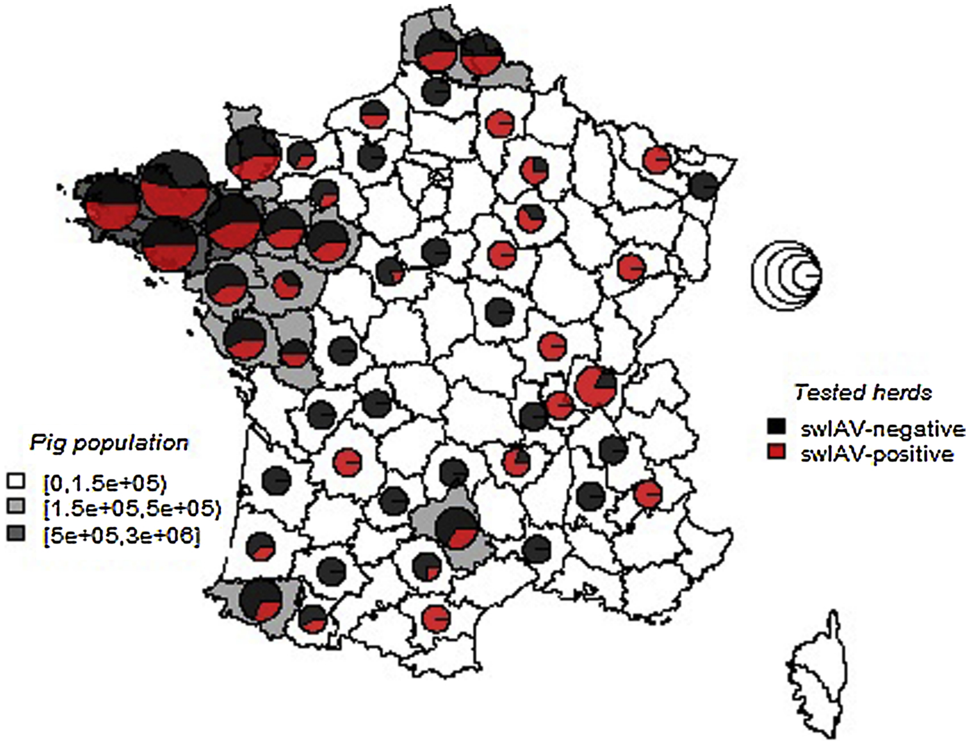

Fig. 2. Geographical distribution of herds visited in mainland France by RESAVIP from April 2011 to March 2018 at the administrative region level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Each circle shows the distribution of IAV-negative (in black) and IAV-positive (in red) pig herds. The four different circle sizes represent the numbers of visited herds within the study period: [1; 10[, [10; 100[, [100; 500[and ≥500 from the smallest to the largest, respectively. The administrative regions are colored in grey gradient according to the size of their pig population, based on the data provided by the National Agricultural Census in 2010 divided into 3 classes.

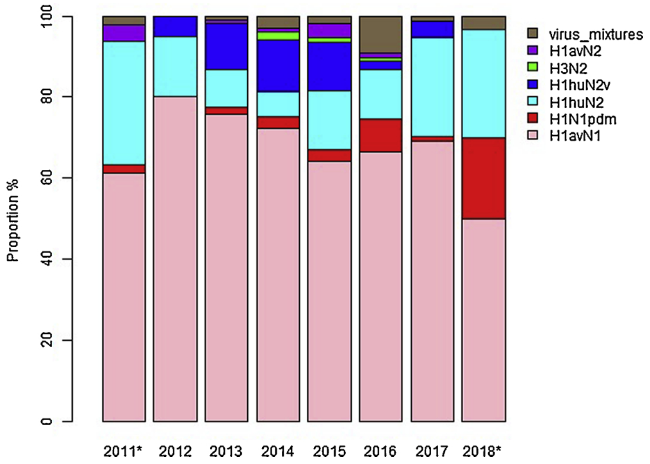

Fig. 3. Distribution of annual proportions of the different swIAV lineages identified by RESAVIP from April 2011 to March 2018.
Pink bars represent the proportion of $H1_{av}N1$ viruses, red bars are H1N1pdm, light blue bars are $H1_{hu}N2$, dark blue bars are $H1_{hu}N2$ variant, green bars are H3N2, purple bars are $H1_{av}N2$, and brown bars are virus mixtures. 2011 and 2018 are incomplete years, with 9 and 3 months only, respectively.

8.2 % (8/98) in 2016 (Fig. 3). Since April 2011, 16/24 (66.7 %) H1N1pdm strains were detected from months December to March, leading to a significantly higher proportion of H1N1pdm strains in winter, i.e. during the seasonal epidemic in humans (3.9 % versus 1.4 %, $p < 0.001$). In January to March 2018, they even accounted for 20 % of the virus strains (6/30) identified by RESAVIP during this quarter. The H1N1pdm virus was identified in 16 administrative regions distributed from the North to the South of the country (Fig. 4). However, its proportion among swIAVs was higher in administrative regions with small pig population sizes (25 %) than in those with a medium-sized (6.9 %) or large population (0.6 %) ($p < 0.001$).

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

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

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

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

Around a quarter of detected swIAVs (218/887) remained partially subtyped (HxN? or H?Ny) or could not be identified (H?N?). Around 60

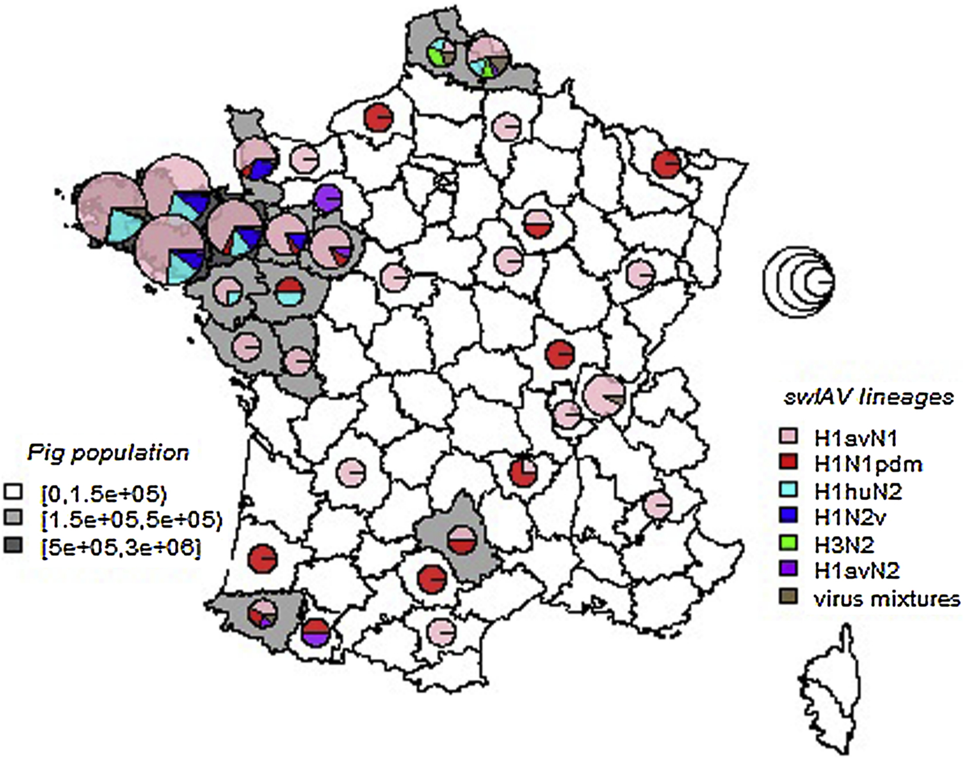

Fig. 4. Relative frequencies of the different swIAV lineages identified by RESAVIP in each administrative region of mainland France from April 2011 to March 2018. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Each circle represents the distribution of identified swIAVs according to their genetic lineages: H1$_{av}$N1 are colored in pink, H1N1pdm in red, H1$_{hu}$N2 in light blue, H1$_{hu}$N2 variant in dark blue, H3N2 in green, H1$_{av}$N2 in purple, and virus mixtures in brown. The four different circle sizes represent the numbers of swIAVs identified within the study period: [1; 10[, [10; 50[, [50; 100[and [100; 300[from the smallest to the largest, respectively. The administrative regions are colored in grey gradient according to the size of their pig population, based on the data provided by the National Agricultural Census in 2010 divided into 3 classes.

% of these samples with unknown viruses (131/218) were automatically excluded from subtyping analyses because exhibiting M gene Cq-values > 35, that indicated an apparent very low genomic load. Among other unknown viruses, 31% (68/218) had a 30 < Cq-value < 35 (data not shown). Finally, only 9 % (19/218) of unknown viruses had a M-gene Cq < 30. In these cases, subtyping failure could have been due to bad virus genome integrity or lack of HA and/or NA RT-qPCR specificity, as only one mutation in primers or probe binding regions may prevent amplification.

3.3. Characteristics of the sampled pig population

Many questions about the visited herds and the sampled pigs were asked in the SADs. Some new items were added subsequently and were therefore included in the SAD only from 2013 onwards; they accumulated less data than other items (Tables 1–5). All pig production types encountered in France were sampled (data not shown), but 76.6 % of visited farms (1,395 cases out of 1,821) were farrow-to-finish herds. SwIAV-positive pigs were detected in all herd types, regardless of the

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

Variable	Total number of available data	Number of respiratory outbreaks of moderate intensity (%)	Number of respiratory outbreaks of high intensity (%)	*p*-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	

[a] data only available from January 2013 to March 2018.
[b] GEE model.
[c] Fisher's Exact test.

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

Variable	Total number of available data	Number of classical epidemiological patterns (%)	Number of recurrent epidemiological patterns (%)	*p*-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 subtype				
$H1_{av}N1$	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	

[a] GEE model.
[b] farrowing-to-weaning + farrowing-to-finishing + farrowing-post weaning.
[c] finishing + post weaning-finishing + post weaning.
[d] data only available from January 2013 to March 2018.
[e] Fisher's Exact test.

presence or absence of breeders on site ($p = 0.27$) (Table 2).

During the study period, only one inactivated trivalent vaccine directed against the three enzootic European lineages $H1_{av}N1$, H3N2, and $H1_{hu}N2$ (Respiporc Flu®3, IDT Biologika GmbH, Dessau-Rosslau, Germany) was authorized in France. Within the RESAVIP framework, it was applied to breeders exclusively. Thus, vaccination was applied constantly to 35.5 %–50.0 % of the monitored breeding population yearly ($p = 0.05$) (Table 1). The probability of detecting a swIAV in ILI pigs was not different in vaccinated and non-vaccinated herds ($p = 0.09$) (Table 2).

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

Both growing pigs and breeders exhibited ILI, but growing pigs were more frequently statistically associated with swIAV detection than gilts and sows (52 % versus 26 %) ($p < 0.001$) (Table 2). The frequency of swIAV-positive cases was higher when ILI-affected animals were piglets under 10 weeks of age, as compared to older animals (51.6 % [≤6

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

Variable	Estimate	Standard Error	OR (95%CI)	*p*-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

[a] GEE model.
[b] data only available from January 2013 to March 2018.

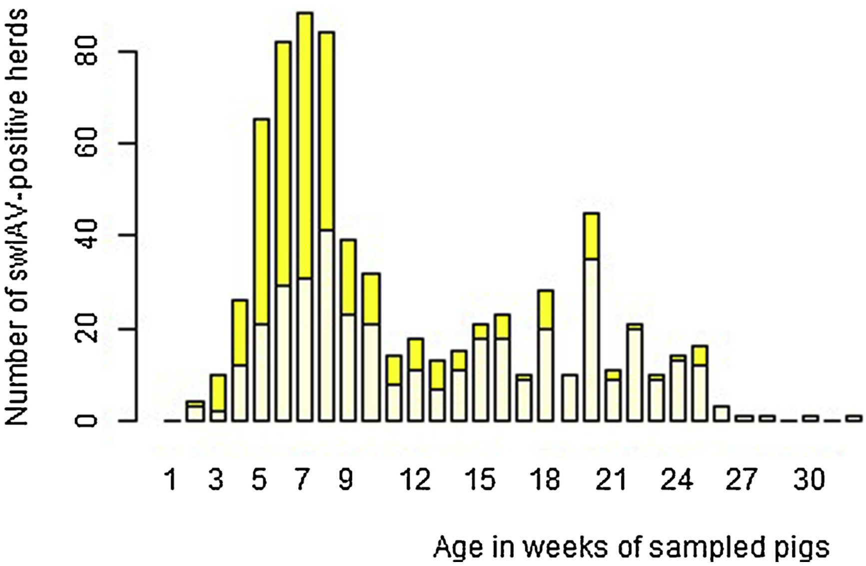

Fig. 5. Distribution of growing pig ages in swIAV-positive herds depending on the epidemiological pattern associated with the influenza-like illness at sampling, i.e., classical (light yellow bars) or recurrent (yellow bars). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

weeks] and 56.1 %]6;9 weeks] versus 46.8 % [> 12 weeks], $p = 0.001$). Animals with hyperthermia (rectal temperature > 40.5°C) were more frequently detected swIAV-positive than animals without fever (52 % versus 43.8 %) ($p = 0.02$) (Table 2).

3.4. Clinical signs and epidemiological patterns associated with swIAV infections (univariate analyses)

For most of the confirmed swIAV-positive cases, veterinarians indicated that pigs exhibited clinical signs of moderate intensity, i.e., lasting no more than 2–3 days at the individual level and without mortality. However, up to approx. 30 % of swIAV-positive cases were reported yearly to have shown severe clinical signs, i.e., lasting longer than usual and/or with some associated mortality (Table 1). In 2011 and 2012, the annual frequency of outbreaks of high-clinical intensity were lower as in following years, probably due to a lack of awareness among veterinarians towards this variable. However, since 2013, this frequency appeared stable from one year to the next, ranging from 17.9 % to 29.5 % ($p = 0.63$) (Table 1). The proportion of cases with high intensity clinical signs was higher from December to March than from April to November (24.5 % versus 18.9 %, $p = 0.03$) (Table 3). High intensity ILI affected all animal types, regardless of their physiological stage or age. However, when considering the different age groups within growing pigs, it appeared that piglets infected before or at 6 weeks of age were at a lower risk of exhibiting clinical signs of high intensity than older pigs ($p = 0.006$) (Table 3). No significant association was found between either the intensity of clinical signs and the swIAV subtype ($p = 0.3$), or between the intensity of clinical signs and the vaccination program applied in the herd ($p = 0.11$) (Table 3).

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

3.5. Multivariate assessment of variables related to swIAV herd positivity, clinical signs and associated epidemiological patterns

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

4. Discussion

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

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

SwIAVs from the four European enzootic lineages were identified, in descending order of proportions, $H1_{av}N1$, $H1_{hu}N2$, H1N1pdm, and H3N2. $H1_{av}N2$ reassortants and several virus mixtures were also found. These results are in accordance with and complementary to those obtained by the NRL through other sampling sources, e.g., requests for diagnostic analyses or epidemiological studies, with the exception of $H1_{hu}N1$ reassortants that were isolated twice in 2011 and 2012 (Simon et al., 2013). While $H1_{av}N1$ strains were detected all around the country irrespective of pig population size, as in many other European countries (Watson et al., 2015), $H1_{hu}N2$ and its antigenic variant $H1_{hu}N2v$ were detected only in the north-western part of France for many years, until $H1_{hu}N2$ detection in the North from 2016. Although the proportion of $H1_{hu}N2v$ declined in recent years, this variant could still be circulating, but ongoing monitoring will give more information on its survival. In Italy, a similar variant seems to have disappeared after it was observed for several years (Moreno et al., 2013). Over the study period, it appeared that H1N1pdm viruses did not establish in the north-western part of the country, the area with the largest pig population size, possibly due to competition with other previous enzootic viruses that were very highly prevalent in this region (Chastagner et al., 2018). By contrast to other countries, reassortants exhibiting H1pdm or N1pdm genes were not detected in France until March 2018 (Krog et al., 2017). However, virus mixtures containing H1N1pdm genes were observed, demonstrating co-infection events; thus, new reassortants may be expected in the future. After the disappearance of H3N2 in the North-west at the end of the 1990′s, this swIAV was detected in herds located in the North in 2012 (Hervé et al., 2012), and RESAVIP confirmed its presence close to the Belgian border, probably in connection with the import of infected animals. Thus, the surveillance network has highlighted increasing swIAV diversity in this area in the last few years. Co-circulations increase the probability of co-infections and reassortment events. RESAVIP identified $H1_{av}N2$ reassortants in several regions, mostly in the western part of the country for years, but also in the North in 2016 (Bonin et al., 2016). More surprising was the identification of $H1_{av}N2$ viruses in the south-western part of France, where enzootic HxN2 viruses had never been detected before, leading us to hypothesize that these reassortants were introduced *in toto* by animals coming from other French regions or European countries (Bonin et al., 2016; Watson et al., 2015). To date, the $H1_{av}N2$ viruses do not appear to be novel enzootic viruses that would circulate within the French pig population, but this could occur, as previously observed in Denmark and the United Kingdom for example (Simon et al., 2014). Therefore, their potential propagation will need to be monitored closely.

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

The data accumulated during the seven years of monitoring did not reveal any impact of month or season on the proportion of swIAV-positive cases, regardless of the virus subtype. This was also observed in closed intensive herds in Canada (Poljak et al., 2014). To date, a cyclical pattern of swIAV circulation in pigs has only been demonstrated on breed-to-wean farms in the United States, through a 5-year study (Chamba Pardo et al., 2017). Two peaks of swIAV-positive cases, whether associated with clinical disease or not, were distinguished in each early winter (December) and late spring (May). They were hypothesized to relate to variations in air humidity and temperature, in line with IAV survival in the environment and patterns of epidemics in humans (Rambaut et al., 2008). In intensive herds in France, the fact that swIAV infections occur all year round could be explained by stable environmental conditions in closed buildings, whereas seasonal occurrence of potential asymptomatic infections cannot be ruled out; however, these types of silent infections were not investigated in the RESAVIP passive surveillance program. Nevertheless, when discriminating swIAV infections depending on the virus lineage, it appeared that the proportion of detected H1N1pdm viruses significantly increased in the winter months, especially over the most recent years and during seasonal influenza epidemics when H1N1pdm was the dominant IAV infecting humans, as in 2016 for example. Thus, *de novo* transmissions from humans to pigs probably occurred during these periods, alongside intra-species H1N1pdm circulation since 2010 (Chastagner et al., 2018; Watson et al., 2015). This is consistent with phylogenetic analyses that estimated many inter-species transmission events since the pandemic (Chastagner et al., 2018; Nelson et al., 2012). The concurrent decrease in detected $H1_{av}N1$ viruses in the same months could be related to potential competition between both H1N1 subtypes in winter, but not outside the human seasonal epidemic when infection pressure is lower. However, further investigations and continued monitoring would be necessary to confirm this hypothesis, as well as to understand the higher proportion of $H1_{hu}N2$ in winter.

Three quarters of visited farms where ILI pigs were sampled were farrow-to-finish herds, while this production type accounts for approximately 36.5 % of pig herds only, according to the national swine identification database (BDporc). However, no statistical link was found between swIAV detection and the presence of breeders in the herd. Interestingly, recurrent infections affected preferentially piglets in nursery, consistent with previous longitudinal studies (Rose et al., 2013). From the data obtained in this study, we also found that the recurrent swIAV infection form was related to the vaccination of breeders in herds. This could be in line with experimental and field studies that pointed out a potential adverse effect of maternally-derived antibodies (MDAs) initially thought to protect the piglets at least at the clinical level (Deblanc et al., 2018; Loeffen et al., 2003). In fact, whereas animals infected in the presence of passive immunity were shown to be less infectious than piglets without MDA, they still shed infectious viral particles with an extended spread of the disease at the batch level, a phenomenon that would contribute to the persistence of the pathogen within the farm (Cador et al., 2016; Rose et al., 2013). However, from the surveillance data, no causal relationships between recurrent infections and vaccination can be ascertained, as it cannot be excluded that vaccination is motivated by recurrent ILI.

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

No statistical relationship between the virus subtype and the intensity of the clinical signs was found, whereas field observations and experimental studies have suggested that the H3N2 and H1N2 viruses were more pathogenic than viruses of the H1N1 subtype (Deblanc et al., 2012; Krog et al., 2017; Zhou et al., 1999). No statistical link was established between the virus subtype and the epidemiological pattern of the disease. A longitudinal survey suggested that recurrent infections could favour concomitant swIAV infections (Rose et al., 2013), but here the virus mixtures could not be related to this epidemiological form, given the surveillance design with independent event-based visits.

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

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.108477.

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