Polymorphism of the *alpha-1-fucosyltransferase* (*FUT1*) gene in several wild boar (*Sus scrofa*) populations in France and link to edema disease

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Abstract

Background: In 2013, an outbreak of edema disease in a population of wild boars (Sus scrofa) took place. This was the first described case as reported worldwide . An enterotoxigenic *Escherichia coli* (presenting the Stx2e and F18 virulence factors) is the main pathogen for this disease in wild boar. The alpha-1-fucosyltransferase gene (*FUT1*) has been identified as the gene regulating the expression of the receptor for *E. coli* stx2e F18 bacteria in domestic pigs affected by the disease. The genotypic frequencies of the *FUT1* gene in European wild boars have not yet been investigated. The genotypes of wild boars for this gene were determined in four French departments with or without edema diseases cases.

Results: All of the wild boars analysed had a genotype susceptible to the disease (GG or AG). The recessive, resistant A allele was found for the first time in wild boars, but in a very small proportion of individuals (7/222). No statistical differences were found between healthy hunted wild boars versus wild boars found dead by edema disease or among the four French departments.

Conclusions: These results suggest that further mortality due to edema disease remains possible in wild boars in France.

Introduction

In July 2013, an abnormal mortality wave in wild boars (*Sus scrofa*) was detected in the department of Ardèche in France (ten individuals found dead in the same commune over a period of 15 days). These wild boars presented distinct neurological disorders. Following numerous analyses (autopsy, bacteriology, toxicology, histology) and the discovery of an enterotoxigenic *Escherichia coli* stx2e F18 belonging to serogroup O139K82, edema disease emerged as the only explanation for this unusual mortality wave. The disease continued to

progress in Ardèche from July 2013 to December 2013, with 109 cases of suspect deaths in 45 communes in central Ardèche [1]. Starting in 2014, the detection of suspect cases evoking the disease began to decrease year by year. However, in 2016 a second outbreak occurred in France. Seventy-five wild boars were found dead in the Albères mountain range in the department of Pyrénées-Orientales. Bacteriological and histological analyses led to the diagnosis of edema disease [2]. The wild boars that were found dying and infected by edema disease presented similar neurological clinical signs, including paddling movements, ataxia, convulsions and trembling, as well as impairments such as transient swelling of eyelids. They were mainly young animals between 4 to 6-months old, which corresponds to the weaning period in wild boars [1]. To our knowledge, these are the first cases of mortality caused by edema disease in a population of wild suidae. It is therefore of utmost importance to understand the origin of these cases by identifying the underlying genetic risk factors.

The alpha-1-fucosyltransferase gene (*FUT1*) was identified as the gene regulating the expression of the F18 receptor in the host of the bacteria causing ED [3, 4]. A single nucleotide polymorphism (G/A M307 mutation) leading to the Ala → Thr amino acid substitution at position 103 of the protein has been identified in this gene [5]. Studies have been conducted to assess the effects of the three possible genotypes [6, 7]. Following experimental inoculation of *E. coli* F18 serogroup O138 to 14 resistant (AA genotype) and 17 susceptible (AG or GG) pigs, 71.4% of the susceptible individuals developed clinical signs while only 5.9% of the resistant individuals did so [7].The AA genotype thus induces better resistance to infection by ETEC (enterotoxigenic *Escherichia coli*) *E. coli* stx2e F18 while the AG and GG genotypes are more susceptible to infection by *E. coli* stx2e F18, the G allele being dominant in relation to the A allele [8, 9]. The expression of this gene depends on the age of the piglet [10]. Indeed, Bao *et al*. demonstrated in 2012 that its expression is most important at the time of weaning (between 3-6 weeks after birth in domestic pigs) [11].

74 Numerous studies have been conducted to estimate the frequency of the different alleles and
75 genotypes in various pig breeds, particularly Asian and European ones [3, 12–17].
76 Conversely, very few comparable studies have been carried out in wild boar populations [3,
77 18, 19]. Those studies involved only Asian individuals and suggest that the G allele has a
78 frequency of 100% [3, 18, 19]. Two studies concluded that the three genotypes, AA, AG and
79 GG, were represented in European pig breeds with fairly significant frequencies while the
80 majority of Asian pig breeds had genotype GG (susceptible), although low frequencies of
81 genotype AG (susceptible) were detected in several Asian pig breeds. As European domestic
82 pigs originated from the domestication of European wild boars (and Asian domestic pigs
83 originated from the domestication of Asian wild boars), the authors suggested that the
84 resistant allele came from European wild boars [3, 16].

85 In light of the emergence of edema disease in wild boar populations, and with a view to limit
86 its impact on domestic pig populations when there are interactions between pigs and wild
87 boars, it is important to determine the potential genetic susceptibility of wild boar populations
88 as a risk factor for this disease. In the present study, we estimated the frequencies of the three
89 genotypes for the *FUT1* gene in different French wild boar samples from two departments
90 where edema disease has been detected, and from two other departments where the disease is
91 assumed to be absent. The genotype and allele frequencies in wild boars, as well as in
92 different domestic pig breeds, were compared with the literature data. Finally the
93 compatibility of wild boars' genetics with edema disease and consequential epidemiological
94 implications as well as potential factors and mechanisms underlying variation in *FUT1* alleles
95 frequencies among wild and domestic suidae are discussed in light of the results.

96

Materials and methods

Samples

Between 2013 and 2017, 222 samples (ear tissue or spleen) were taken from wild boars (hunted healthy animals n = 178 or animals that died of edema disease n = 44) in four French departments (Figure 1) using opportunistic and targeted sampling.

The auricular samples (ear tips) were taken by hunters in the different departments as well as by technicians from departmental hunting federations. Once collected, the samples were frozen at -20°C to conserve them.

In Ardèche, all of the auricular samples (Figure 2) were collected in areas where there had been outbreaks of edema disease in wild boars. Of these, 41 were from wild boars suspected (or confirmed) to have been affected by edema disease between 2013 and 2015. In 2014 and 2016, samples were collected from respectively 64 and 48 hunted wild boars which presented no sign of the disease.

In the Pyrénées-Orientales, all of the samples (Figure 3) also came from disease-affected areas. These included three spleen specimens taken from wild boars which had died of edema disease in 2016, and 17 ear tissue specimens taken from wild boars which showed no sign of the disease and which were hunted between January and February 2017.

The two departments in which no cases of edema disease have been detected are Lozère (Figure 4) and Hérault (Figure 5). Their wild boar biogeographic and population characteristics are relatively similar to those of Ardèche and Pyrénées-Orientales. They were used as control territories, with 19 ear tissue samples taken from wild boars hunted in 2014 for Lozère, and 30 tissue samples (25 spleen and 5 ear) from wild boars killed during the 2016-2017 hunting season for Hérault.

Edema disease diagnostics in wild boars

Edema disease was diagnosed in wild boars using the following criteria. The location where the diseased wild boar or the wild boar carcass was discovered was considered. A diseased animal or a carcass discovered in the same commune as or in a commune adjacent to a commune where a confirmed edema disease case had already been recorded less than two months before was considered as suspicion of an edema disease case. Clinical signs and lesions recorded at the time of discovery or during the autopsy were also considered. The clinical signs on a live wild boar considered as indicative of an edema disease case were shakings/convulsions, pedalling, ataxia and lateral decubitus. The lesions observed during the autopsy of a dead wild boar considered as indicative of an edema disease case were edema on eye lids or in the mesocolon, thoracic, abdominal or pericardial effusions and congestive haemorrhagic colitis. Bacterial analyses were also undertaken on the content of the digestive tractus. The isolation and identification of O139k82 or O141k85 *Escherichia coli* was considered as a confirmation of an edema disease case. Finally, histological analyses were undertaken to detect neuronal vacuolisation which was also considered as a confirmation of an edema disease case. Table 1 shows the different combinations of criteria that lead to strong suspicions or confirmations of edema disease cases.

Table 1. Number of samples for each analysis

Edema disease diagnostics	Sample
Histological and bacteriological analyses, clinical signs, location of wild boar corpses	6/44
Histological analyses, clinical signs, location of wild boar corpses	1/44
Bacteriological analyses, clinical signs, location of wild boar corpses	18/44
Clinical signs, location of wild boar corpses	19/44

Genotyping

DNA was extracted using the Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany). The polymorphism of the *FUT1* gene was then determined using PCR (polymerase chain reaction). The primers F 3'-TGCATGGCAGGCTGGATGAAG-5' and R 3'-CCAACGCCTCCGATTCCTGTC-5' were used as the sequence coding for the sequence of the gene FUT1of GenBank. Amplification by PCR (final volume = 50 µl) was done using 25 µl of taq polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 1 µl of each primer at 10 µM, and 22 µl of water, with 1 µl containing approximately 200 ng of DNA. The PCR conditions were: 94°C for 3 minutes, followed by 50 cycles (at 94°C for 1 minute, 53°C 1 minute, 72°C 1 minute), and then 72°C for 3 minutes. The PCR products were then purified by migration on gel electrophoresis (2% agarose + 7 µl of SYBR Safe stain for 100 ml of gel). After migration, the purified DNA was extracted using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). The purified DNA was then sequenced by the GATC laboratory. The obtained sequences were read using the Chromas lite software. For the susceptible allele G sequence was CCTG*G*CGCAG while the resistant allele A, it sequence was CCTG*A*CGCAG.

Bibliographic synthesis

A bibliographic synthesis was undertaken to obtain the maximum amount of data on the frequencies of different genotypes and alleles of the *FUT1* gene (Appendix). This bibliographic synthesis was conducted using Google Scholar, PubMed, and ScienceDirect search engines. The key words used were (i) pig, (ii) wild boar, (iii) FUT1 and (iv) alpha-1-

fucosyltransferase. More specifically, the query was: ("pig" or "wild boar") and ("FUT1" or "alpha-1-fucosyltransferase"). Only articles that were in English and presenting genotype and allele frequencies in the populations studied were retained. The geographic origins of different pig breeds were then identified and classified in three main groups: America (combining Central and North American pigs), Europe and Asia. The same procedure was used for wild boar; with Asian and Russian wild boars regrouped under the name Asian wild boar.

Statistical analyses

Variations in genotype and allele frequencies in wild boar populations in France

The null hypothesis of frequency homogeneity (genotype or allele) between the departments where wild boars were sampled in France was tested by applying Fisher's exact test to contingency tables displaying geographical origin and genotype or allele. This same test was used to test the null hypothesis of frequency homogeneity between French wild boars suspected of being infected by edema disease and other French wild boars that are *a priori* not infected.

Variations of allele frequencies between different types (wild boar and various breeds of domestic wild boar) of *Sus scrofa*

In addition, a more comprehensive analysis was undertaken of variations in allele frequencies that combined the data collected in French wild boar populations with data from the literature on allele frequencies of other wild boar populations and of different domestic pig breeds. To

do this, a generalized linear model (GLM) was fitted in which the binomial response variable was the frequency of the A allele (number of A alleles relative to the total number of typed alleles). The GLM included the fixed effect of a categorical variable with 5 modalities (European or Asian wild boars, and American, Asian, or European domestic pigs). In this model, each combination of breed and origin (each line of the table in the annex) was considered as a statistical unit. A post-hoc Tukey test was then performed to make pairwise comparisons between the different categories. The statistical analyses were conducted using R software, and more precisely with the "multcomp" package for the post-hoc Tukey test.

Results

Polymorphism of the FUT1 gene in different French wild boar populations (experimental data)

The digestion of the PCR products produced fragments of 109 nucleotides. The genotype and allele frequencies in the samples studied are presented in Table 2 below.

Table 2. Genotype and allele frequencies of the FUT1 gene in different wild boar populations in France

Sample location	Suspect ED / hunted	Sample size	Genotype frequency (sample size)			Allele frequency	
			AA Resistant	AG Susceptible	GG Susceptible	A Resistant	G Susceptible
Ardèche	ED	41	0 (0)	0.049 (2)	0.951 (39)	0.024	0.976
	Hunted	112	0 (0)	0.027 (3)	0.973 (109)	0.014	0.986
Pyrénées-Orientales	ED	3	0 (0)	0 (0)	1 (3)	0	1
	Hunted	17	0 (0)	0 (0)	1 (17)	0	1
Lozère	Hunted	19	0 (0)	0 (0)	1 (19)	0	1
Hérault	Hunted	30	0 (0)	0.067 (2)	0.933 (28)	0.033	0.967

France (total)	ED / Hunted	222	0 (0)	0.032 (7)	0.968 (215)	0.016	0.984

ED : Edema disease

The resistant AA genotype was not detected in any of the boars sampled. AG heterozygotes were detected at a low frequency in only two departments (5/153 in Ardèche and 2/30 in Hérault), whereas the animals from the other two departments only had the homozygous GG genotype. The GG genotype thus is largely predominant in the wild boars sampled. The frequencies of the different genotypes of the *FUT1* gene of the wild boars sampled in France do not vary significantly according to the department (Fisher's exact test $p>0.05$). These frequencies also do not vary significantly according to the animal's status with regard to edema disease (test limited to the samples from Ardèche and Pyrénées Orientales, Fisher's exact test $p>0.05$).

The frequency of the resistant A allele therefore is very low among the sampled European wild boars (0.016) irrespective of the edema disease status and the department.

Comparison of allele frequencies between pig and wild boar breeds of different origins (synthesis of data from the literature – Appendix)

The averages of the allele frequencies appear to be different depending on the origin of the animals (Table 3).

Table 3. FUT1 allele frequencies for pigs and wild boar breeds of different origins

	American pig (n = 422)	European pig (n = 2874)	Asian pig (n = 2316)	Asian wild boar (n = 136)	French wild boar (our study)

					(n = 222)
A allele	0.333	0.245	0.020	0	0.016
G allele	0.667	0.755	0.98	1	0.984

The GLM model shows that the allele frequency depends on the origin of the animals. Indeed, the explanatory variable "Origin" is highly significant (Table 4). Only the difference in allele frequency between French wild boars and Asian wild boars is not significant. However, due to the fact that no allele A was detected for the Asian wild boar category, the estimate of the parameter associated with this category is strongly negative on the logit scale with a very large standard error. This well-known artefact for parameters estimated near the bounds (0 or 1) in GLMs compromises the validity of statistical comparison tests with this category. However, the frequency of the resistant A allele is lowest in Asian wild boars, followed by French wild boars and Asian pig breeds, and then European pig breeds. The frequency of the resistant A allele is the highest in American domestic pig breeds.

Table 4. Results of the GLM model

	GLM(A~origin), family=binomial		
	Estimate	**Std.error**	**P-value**
French wild boar	Reference		
European pig	2.970	0.018	$<2.2 \times 10^{-16}$ ***
American pig	3.602	0.020	$<2.2 \times 10^{-16}$ ***
Asian wild boar	-14.339*	32.285	0.657
Asian pig	1.400	0.020	$<2.2 \times 10^{-16}$ ***

These results were confirmed using the post-hoc test (Table 5), which enabled us to refine the comparisons of allele frequencies between the different origins of domestic pigs and wild boars.

Table 5. Results of the post-hoc test

	French wild boar	**Asian pig**	**European pig**
Asian pig	1.400±0.019***	---	
European pig	2.970±0.018***	1.570±0.006***	---
American pig	3.601±0.020***	2.202±0.009***	0.632±0.007***

Estimate±Std.error *** p<0.001

Discussion

Wild boar genetics compatible with the emergence of edema disease?

The very low frequency of the A allele (0.016) in the wild boars sampled in four French departments is in line with the results reported in the literature for Asian wild boars [3, 18, 19]. These results suggest that wild boars are susceptible to edema disease, yet no case of mortality due to this disease had been recorded prior to the episode reported in France in 2013. It is possible that mortality caused by this disease existed without being detected, or that it was under-diagnosed. In France, for example, certain group mortality events in wild boars remain unexplained (personnal communication: SAGIR). An alternative hypothesis explaining the absence of documented cases of edema disease in wild boars is a recent exposure to enterotoxigenic *E. coli* stx2e F18. If indeed these strains come from domestic pigs, the rapid increase in wild boar populations in France [20] and the rising number of French open-air pig farms [21] may have enabled an increase in direct and indirect contacts between pigs and wild boars, thereby favouring the passage of different pathogens between the domestic and wild compartments of this same species (*Sus scrofa*). Another hypothesis explaining the emergence of this disease in wild boars would be a change in the bacteria's pathogenic mechanism. If the bacteria was able to multiply without needing to adhere to intestinal epithelial cells or using another receptor, the genetic risk factor would no longer affect the emergence of edema disease in wild boars.

Although Asian pig breeds do not present (or present at a very low frequency) the *FUT1* genotype conferring resistance to edema disease, the susceptibility of these animals to post-weaning diarrhoea appears to be lower than that of western pig breeds [16]. One therefore may hypothesize that one or more other genes in the gene black box modulate the susceptibility of pigs to edema disease [22]. The emergence of edema disease in wild boar populations in France could then be related to a hypothetical increase in the frequency of wild boar/domestic pig hybrids, leading to an increased susceptibility to this disease.

Genetic modification in subsequent generations of wild boar is possible following the death of susceptible wild boars. A future study is needed to clarify this point.

Difference of allele frequency between wild boars and domestic pigs

Frequency of the A allele and domestication of pigs

Domesticated pigs have two main origins: Europe and Asia. European domestic pig populations are the result of the domestication of European wild boars, while Asian pigs originate from Asian wild boars [23, 24]; there is a deep phylogenetic split between European and Asian wild boars [25]. Moreover, the frequencies of the resistant A allele in different Asian and western pig breeds already have been compared by Yan *et al.* (2003) and Bao *et al.* (2008) using samples obtained in pig farms located in China [3, 16]. These studies show that the resistant A allele is much more frequent in European and American breeds than in Asian breeds, a finding confirmed by other studies identified in our bibliographic synthesis. The

284 authors of these works deduce from these results that the resistant allele likely came from
285 European wild boars [3] from which western domestic pig breeds have descended [23, 26, 27].
286 According to this phylogenetic pattern and these previous results we would have expected that
287 the frequency of the resistant A allele in the wild boars sampled in France would be fairly
288 similar to the frequency of the resistant A allele in European domestic pig breeds and would
289 be substantially higher than the frequency of the resistant A allele in Asian wild boars and
290 Asian domestic pig breeds. However, our results suggest that the frequency of the resistant A
291 allele is much lower in French wild boars than in European domestic pig breeds and fairly
292 similar to that in Asian wild boars, and Asian domestic pig breeds. Several hypotheses could
293 explain this pattern.

294 The analysis of the genomes of European and Asian wild and domestic pigs by Frantz *et al.*
295 (2015), raised possible explanation related to the evolutionary and demographic history of
296 European wild boars and European domestic pigs populations. Indeed their results suggest
297 that European wild boar population experienced strong bottlenecks due to overhunting and
298 habitat loss [28]. Such demographic bottlenecks are suspected to result, through the associated
299 genetic drift, in changes in the genetic composition in wild boar populations including the loss
300 of alleles [29]. The resistant A allele could have been lost during such demographic
301 bottlenecks. Another interesting hypothesis presented by Frantz *et al.* (2015) is that some wild
302 boar population that contributed to the current genetic pool of European domestic pigs are
303 extinct [28]. The resistant A allele could originate from such extinct populations. Under these
304 hypotheses, the few wild boar individuals (7/222) with this A allele could be the products of a
305 (more or less recent) hybridization between pigs and wild boars.

306 Another scenario could be envisioned in which the frequency of the A allele would be very
307 low or even null in the wild boar populations from which European domestic pigs originate.
308 Under this hypothesis the A allele would have appeared and/or been selected in domestic pig

populations following domestication. It has been demonstrated in a population of Sutai breed (Asian) pigs that between 2008 and 2011 the frequency of the A allele increased. The authors of this study also examined the relationship between *FUT1* gene polymorphism and growth and found that pigs with the AA genotype (resistant to edema disease) had the best growth. The authors of this study therefore suggest that the increased frequency of the A allele is the consequence of artificial selection aimed at not only improving resistance to post-weaning diarrhoea and edema disease, but also production performance [30]. Another study [31] examined the association between the genotype for the *FUT1* gene and litter size. In this study, animals with the AG genotype had better group performance and larger litter sizes than those with the GG genotype (the number of individuals with the AA genotype was too small to be analyzed [31]). Filistowicz and Jasek also studied the effect of the *FUT1* gene on fertility and reproductive success rates, but by looking at the interactions between the polymorphisms of the *FUT1* [32] and *MUC4* (gene associated with the receptors of bacteria responsible for neonatal diarrhoea) genes [33]. They detected a positive interaction between the $MUC4^{B/B}$ and $FUT1^{A/G}$ genotypes on fertility and a negative interaction between the $MUC4^{A/A}$ and $FUT^{A/G}$ genotypes on fertility [32]. In these studies, the association between the *FUT1* gene, fertility, and animal production performance is described but incompletely understood. By considering the hypothesis of the emergence and then selection of the A allele in some domestic pig populations, the few wild boar individuals (7/222) with this A allele could again involve a (more or less recent) hybridization between pigs and wild boars.

Frequency of the A allele and pig-wild boar interface in France

Numerous possibly hybrid wild boars have been observed in Ardèche in the communes where the samples were taken. These wild boars are suspected of being ‘hybrids’ due to their

phenotypic characteristics: white tips of the legs, spotted coats, thick layer of fat, drooping ears, litters of over 10 piglets. In addition, chromosomal screening of French wild boars conducted on breeding farms and in different natural wild boar populations has demonstrated the presence of hybrids (2n = 37 or 2n = 38, whereas the Western European wild boar has 2n = 36 chromosomes), sometimes at high frequencies [34–36]. Several complementary studies could be set up to corroborate the hypothesis of the ancestral nature of the G allele and of the link between domestication and the emergence of the A allele. It could then be possible to genotype non-hybridized wild boars (2n = 36 chromosomes), in natural populations of wild boars considered to be "purebred" (identified as purebred through a follow-up study of the karyotype of wild boars) or in breeding farms historically free of hybrids to confirm the absence of the A allele when there is no introgression with domestic pigs. It also would be possible to investigate variations of allele frequencies of the *FUT1* gene along frequency gradients of pig-wild boar interactions. Lastly, it would be interesting to monitor on a longitudinal basis the rate of evolution of allele frequencies of the *FUT1* gene in wild boar populations (following the protocol used with the Sutai breed [30]).

Allele frequency and wild boar hunting pressure

The evolution of hunting practices and of wild boar populations is enabling a strong renewal of wild boar populations. It is possible that a selection of individuals with rapid growth, and therefore with an ability to reproduce increasingly younger, is taking place. Indeed, to enable wild boar populations to increase, some hunting organizations in certain French departments request their hunters to avoid killing female wild boars that have surpassed the threshold weight needed to reproduce. A selection of wild boars consequently is causing an

artificialization of wild boar populations as hunters are allowing the survival of wild boars with higher growth rates. Given that domestic pigs with the A allele would have stronger growth and reproduction rates [30], it is logical to hypothesize that wild boars with an AG or AA genotype also have a stronger growth rate.

Moreover, in the French departments (located in southern France) where sampling was possible, the selection of hunted wild boars is very limited, which contrasts with northern France, where it is much more widespread.

The wild boar, a potential reservoir of the bacterium?

Wild boars, which according to our results predominately have a genotype enabling the adhesion and multiplication of enterotoxigenic *E. coli* stx2e F18 strains responsible for edema disease, could be potential reservoirs of the bacteria. A serological study on pig farms highlighted a seroprevalence of 96.4% for *E. coli* F18 for open-air domestic pigs, and 88.8% for housed domestic pig farms [37]. As the F18 virulence gene is one of the virulence genes identified for the bacterium found in wild boars, an equivalent study on wild boars would be useful to anticipate potential mortalities in wild boars in the event that wild boars act as a reservoir of this bacteria. With increasing pig-wild boar interactions, the passage of the bacteria from the wild to the domestic compartment should be considered.

Abbreviations

E. coli : *Escherichia coli*

FUT1 : alpha-1-fucosyltransferase

ETEC : enterotoxigenic *Escherichia coli*

PCR : Polymerase Chain Reaction

GLM : Generalized Linear Model

ED : Edema Disease

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article. Materials (DNA), samples (tissues and spleen) are available from the corresponding author on reasonable request.

Authors' contributions

GP designed and supervised the study. GP wrote the original manuscript. GP, VG, KCM, AD, GPM, DD and AD revised the manuscript. DD has developed the protocol. GP performed the experiments. GP, VG and KCM analysed the data. All authors have reviewed and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Authors declare that they have no competing interests.

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541 **Fig 1. Location of the four French departments sampled**. In blue: Ardèche, in orange:

542 Lozère, in green: Hérault, in red: Pyrénées-Orientales

543 **Fig 2. Location of the sources of samples in Ardèche**

544 **Fig 3. Location of the sources of samples in Pyrénées-Orientales**

545 **Fig 4. Location of samples in Lozère**

546 **Fig 5. Location of samples in Hérault**

547

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549 **APPENDIX**

550

Breed	Origin	Sample size	AA genotype frequency	AG genotype frequency	GG genotype frequency	A allele frequency	G allele frequency	Reference
Eurasian wild boar (*Sus scrofa* L., 1758)	Asia	89	0	0	1 (89)	0	1	[19]
Large White	Europe	174	0.052 (9)	0.448 (78)	0.5 (87)	0.276	0.724	[14]
Pudong White	Asia	168	0.018 (3)	0.405 (68)	0.577 (97)	0.22	0.78	
Large White boars	Europe	48	0.021	0.354	0.625	0.2	NA	[12]
Large White sows	Europe	77	0.026	0.429	0.545	0.24	NA	
Landrace boars	Europe	19	0.211	0.789	0.263	NA	NA	
Pietrain	Europe	171	0.088	0.415	0.497	NA	NA	
Duroc	America	102	0.206	0.48	0.314	NA	NA	
Prestice Black Pied pig	Europe	55	0.836	0.164	0	NA	NA	
Red Mangalitsa	Europe	40	NA	NA	NA	0.69	NA	[13]
Bazan	Europe	62	NA	NA	NA	0.3	NA	
Duroc	America	44	0.136 (6)	0.341 (15)	0.523 (23)	0.307	0.693	[3]
Yorkshire	Europe	62	0 (0)	0.323 (20)	0.677 (42)	0.162	0.838	
Pietrain	Europe	54	0.167 (9)	0.333 (18)	0.500 (27)	0.334	0.666	
Landrace	Europe	56	0 (0)	0.179 (10)	0.821 (46)	0.09	0.91	
Erhualian	Asia	57	0 (0)	0.211 (12)	0.789 (45)	0.106	0.894	
Fengjin	Asia	46	0 (0)	0.084 (4)	0.913 (42)	0.044	0.956	
Meishan	Asia	40	0 (0)	0.025 (1)	0.975 (39)	0.013	0.987	
Huai	Asia	35	0 (0)	0.086 (3)	0.914 (32)	0.043	0.957	
Leping	Asia	35	0 (0)	0 (0)	1 (35)	0	1	
Xiushuihang	Asia	36	0 (0)	0 (0)	01 (36)	0	1	
Wanan	Asia	31	0 (0)	0 (0)	1 (31)	0	1	

Breed	Origin	Sample size	AA genotype frequency	AG genotype frequency	GG genotype frequency	A allele frequency	G allele frequency	Reference
Lingao	Asia	31	0 (0)	0.032 (1)	0.968 (30)	0.016	0.984	
Northeast min	Asia	52	0 (0)	0 (0)	1 (52)	0	1	
Rongchang	Asia	46	0 (0)	0 (0)	1 (46)	0	1	
Songliao	Asia	59	0 (0)	0 (0)	1 (59)	0	1	
Wuzhistan	Asia	50	0 (0)	0 (0)	1 (50)	0	1	
Tibetan	Asia	53	0 (0)	0 (0)	1 (53)	0	1	
Sujiang	Asia	31	0 (0)	0.258 (8)	0.742 (23)	0.129	0.871	
Sutai	Asia	98	0 (0)	0.092 (9)	0.908 (89)	0.046	0.954	
Hybrid	Asia	41	0 (0)	0.463 (19)	0.537 (22)	0.232	0.768	
Asian wild boar	Asia	32	0 (0)	0 (0)	1 (32)	0	1	
Prestice Black Pied pig	Europe	92	0.023	0.233	0.744	0.14	0.86	[15]
Large White	Europe	231	0.05 (11)	0.47 (108)	0.48 (112)	0.28	0.72	[38]
Landrace	Europe	107	0.02 (2)	0.35 (38)	0.63 (67)	0.2	0.8	
Songliao Black	Asia	109	0.02 (2)	0.20 (22)	0.78 (85)	0.12	0.88	
(Large white x Landrace) x Leicoma	Europe	120	0.025	0.292	0.683	0.17	0.83	[23]
Large White	Europe	14	0	0.71	0.28	0.36	0.64	[39]
Landrace	Europe	32	0	0.417	0.58	0.22	0.78	
Zlotnicka Spotted	Europe	8	0.37	0.5	0.12	0.63	0.37	
Zlotnicka White	Europe	12	0	0.438	0.562	0.21	0.79	
Polish Large White x ZS	Europe	18	0.33	0.5	0.17	0.58	0.42	
Duroc	America	205	NA	NA	NA	0.278	0.722	[40]
Large White	Europe	431	NA	NA	NA	0.061	0.939	
Landrace	Europe	794	NA	NA	NA	0.092	0.908	
Duroc	America	43	0.116 (5)	0.465 (20)	0.419 (18)	0.349	0.651	[18]
Landrace	Europe	262	0	0.046 (12)	0.954 (250)	0.023	0.977	
Large White	Europe	40	0.075 (3)	0.425 (17)	0.500 (20)	0.287	0.713	

Breed	Origin	Sample size	AA genotype frequency	AG genotype frequency	GG genotype frequency	A allele frequency	G allele frequency	Reference
Duroc x Landrace x Largewhite	Europe	461	0.086 (39)	0.423 (195)	0.492 (227)	0.297	0.703	
Duroc x wild boar	Europe	33	0.03 (1)	0.394 (13)	0.576 (19)	0.227	0.773	
Largewhite x Jianli	Asia	36	0	0	1 (36)	0	1	
Qingping	Asia	33	0	0	1 (33)	0	1	
petit Meishan	Asia	43	0	0	1 (43)	0	1	
Jinhua	Asia	26	0	0	1 (26)	0	1	
Jianli	Asia	49	0	0	1 (49)	0	1	
Wild pig	Asia	15	0	0	1 (15)	0	1	
French wild boar	Europe	219	0	0.032 (7)	0.968 (212)	0.016	0.984	This study
Yorkshire	Europe	29	0.1	0.45	0.45	0.33	0.67	[17]
Pelon	America	46	0.11	0.5	0.39	0.36	0.64	
Cuino	America	28	0.39	0.32	0.29	0.55	0.45	
Duroc	America	56	0.036 (2)	0.232 (13)	0.732 (41)	0.152	0.848	[16]
Landrace	Europe	58	0.017 (1)	0.121 (7)	0.862 (50)	0.071	0.929	
Large White	Europe	60	0.033 (2)	0.384 (23)	0.583 (35)	0.224	0.776	
Pietrain	Europe	17	0.059 (1)	0.353 (6)	0.588 (10)	0.222	0.778	
Hampshire	Europe	59	0.017 (1)	0.204 (12)	0.779 (46)	0.113	0.887	
Min	Asia	50	0	0	1	0	1	
Mashen	Asia	39	0	0	1	0	1	
Luchuan	Asia	56	0	0	1	0	1	
Tibetan	Asia	60	0	0	1	0	1	
Gogbujiangsa tibetan	Asia	61	0	0	1	0	1	
Bama xiang	Asia	62	0	0	1	0	1	
Kele	Asia	51	0	0	1	0	1	
Dahe	Asia	16	0	0	1	0	1	
Wuzhishan	Asia	60	0	0	1	0	1	
Shanggao	Asia	60	0	0	1	0	1	
Dongxianx spotted	Asia	60	0	0	1	0	1	
Leping spotted	Asia	62	0	0	1	0	1	

Breed	Origin	Sample size	AA genotype frequency	AG genotype frequency	GG genotype frequency	A allele frequency	G allele frequency	Reference
Yushan hei	Asia	61	0	0	1	0	1	
Hang	Asia	61	0	0	1	0	1	
Erhualian	Asia	62	0	0	1	0	1	
Jinhua	Asia	62	0	0	1	0	1	
Ningxiang	Asia	61	0	0	1	0	1	
Rongchang	Asia	60	0	0	1	0	1	
Neijiang	Asia	62	0	0	1	0	1	
Lingao	Asia	62	0	0.258 (16)	0.742 (46)	0.129	0.871	
Guangdong	Asia	60	0	0	1	0	1	

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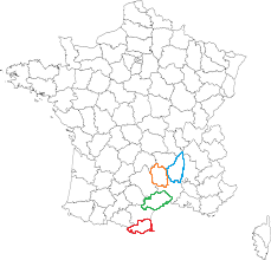

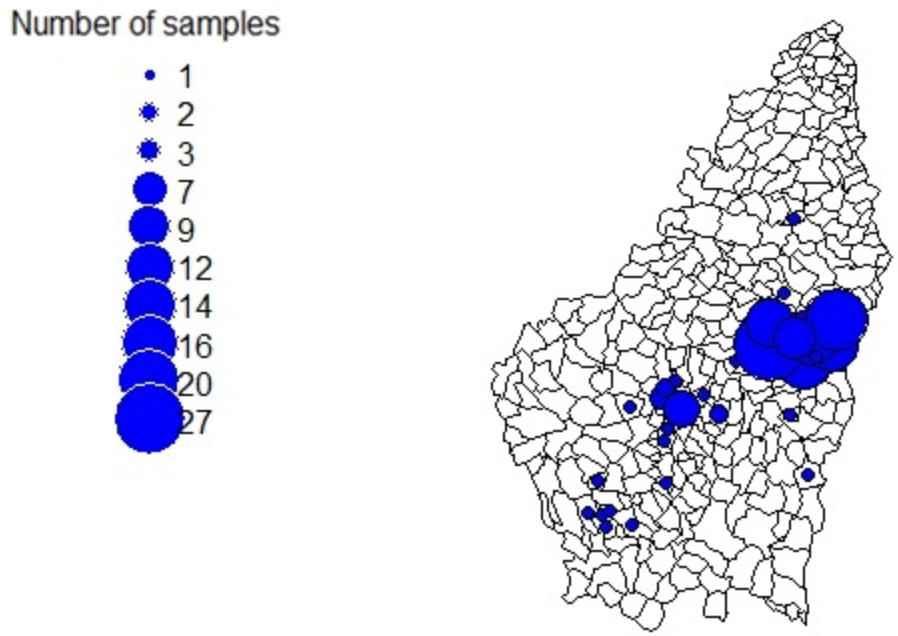
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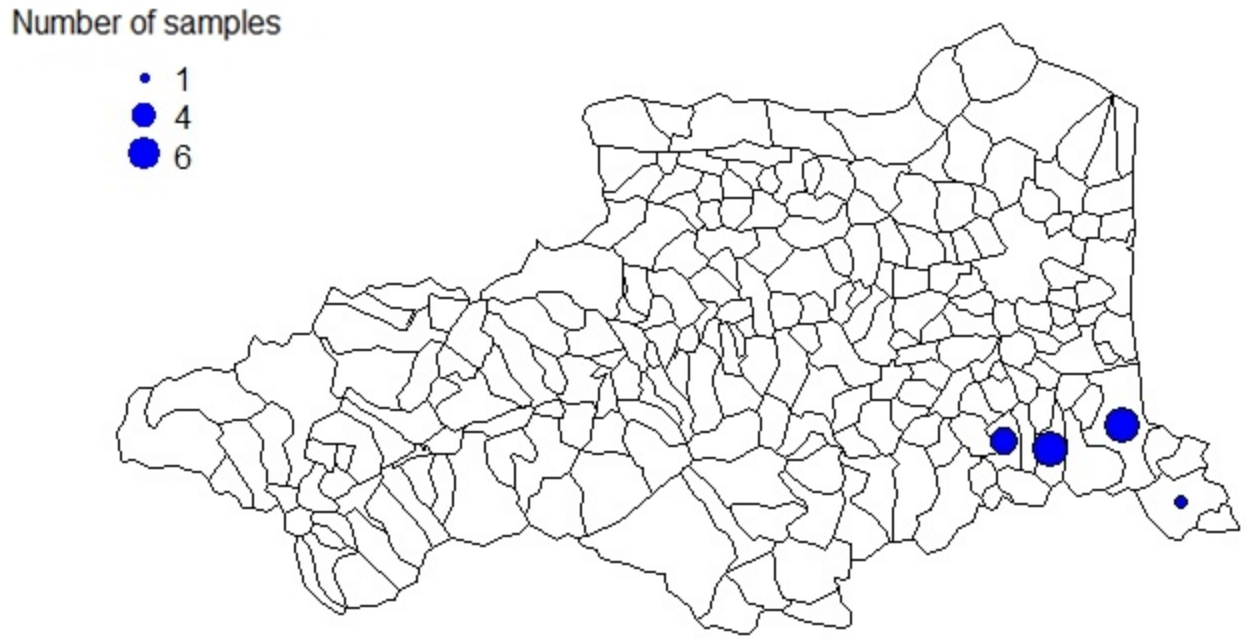
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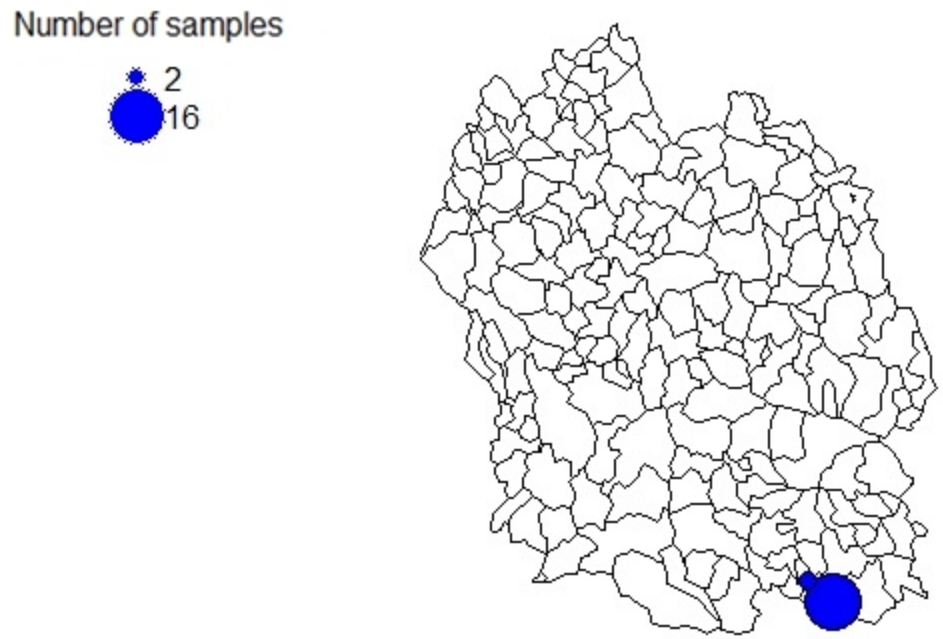
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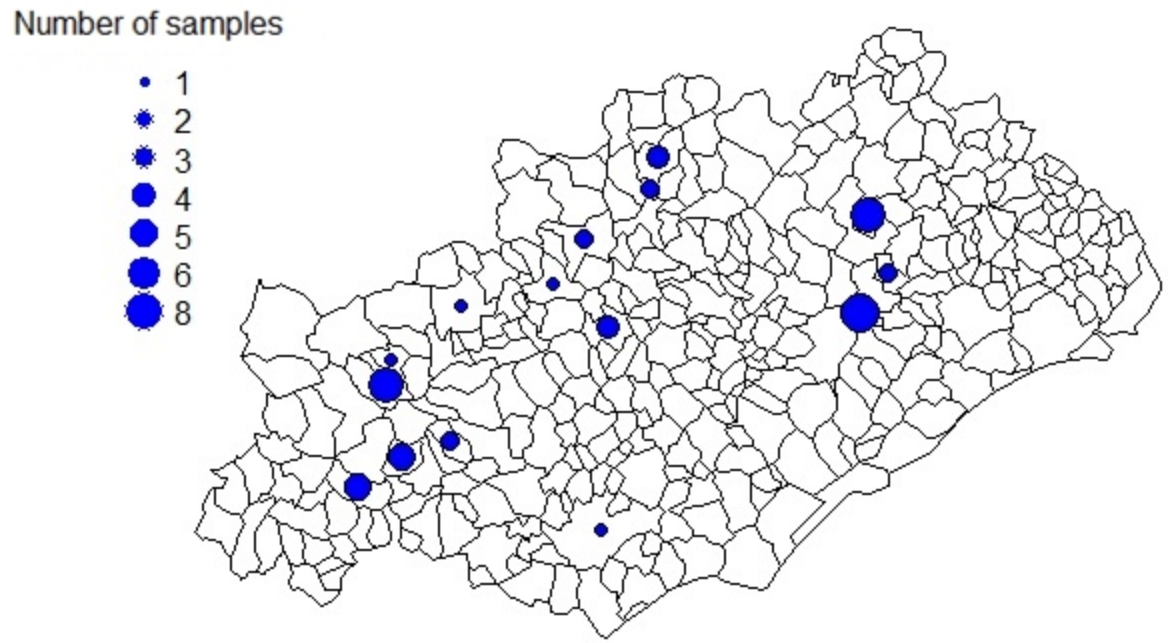
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