

I) Genotyping of codon 129 and western-blot profiles of PrP^{Res}

Supp.Table.1: Proportions of patients for which genotype at codon 129 of PRNP, western-blot (WB) typing of PrP^{Res} or both were performed

Cohort considered (n, %)	Genotype		Western-blot		Both genotype and WB	
All sCJD cases	1667	(68.32 %)	831	(34.06 %)	620	(25.41 %)
All cases with the same birth and death dep.	763	(67.34 %)	382	(33.72 %)	281	(24.8 %)

Data was taken from within the sCJD cases identified in France during the 1992-2016 period and the group of people who have a single department for both birth and death.

II) Equations for the calculations of sCJD incidence per sub-group

The incidence of CJD can be estimated using a simple equation (Eq.1), with the incidence (*inc*) reflecting the observed reality (*obs*) in relation to the national population (*pop*) and the duration of the surveillance (*t*).

$$inc = \frac{obs}{pop \times t} \quad (1)$$

Because the French population varies slightly from year to year, the total of each year between 1992 and 2016 were added together into *popc*, thus simplifying the equation to include the “ $\times t$ ” part of the equation into the variable *popc* to calculate this incidence *i*, which better reflects the true French population over this period of time (1’).

$$i = \frac{obs}{popc} \quad (1')$$

Equation.1: Incidence of CJD from available data, with *i* the incidence rate (in cases per people per year), *obs* the observed cases, *pop* the French population (Insee¹) and *t* the timeframe observed (1992-2016). In the simplified equation (1’), *popc* is the cumulated French population between 1992 and 2016, which includes the *t* variable.

For a finer, more representative measure of this incidence, age and sex were taken into consideration (Eq.2). The observed and reference populations were split into age-sex-specific groups (*obs_{ag & s}* and *popc_{ag & s}* respectively) between men and woman of 20 different age groups each ranging over 5 years of age (i.e. 0-4, 5-9, etc.). The last group exceptionally ranges from 95 years old to 200 to capture the limited number people over 100 years old. These subgroups were then used to measure the age-sex-specific incidences (*i_{ag & s}*).

$$i_{ag \& s} = \frac{obs_{ag \& s}}{popc_{ag \& s}} \quad (2)$$

Equation.2: Age-sex-specific incidence of CJD from available data, with *i_{ag & s}* the age-sex-specific incidence rates (in cases per people per year, sex, and age group), *obs_{ag & s}* the age-sex-specific observed cases, *pop_{ag & s}* the age-sex-specific French population (Insee¹) within the timeframe observed (1992-2016).

The expected cases were then calculated per department in an age-sex-specific manner (*exp_{ag & s}*) from the appropriate incidence rates of the disease (*i_{ag & s}*, Eq.2) and the age-sex-specific population present in this department (*pop_{ag & s}*) throughout the period of interest (1992-2016) (Eq.3), similarly to the way *popc* (Eq.1’) was calculated.

$$exp_{ag \& s} = popd_{ag \& s} \times i_{ag \& s} \quad (3)$$

Equation.3: Measure of each department’s age-sex-specific expected cases (*exp_{ag & s}*) between 1992-2016 from age-sex-specific previously calculate incidence rates (*i_{ag & s}*), and *popd_{ag & s}* the cumulated population in respective department between 1992 and 2016¹, grouped in an age-sex-dependant manner.

The total number of expected cases of CJD (*exp*) for each set of maps was considered to be the sum of all age-sex-specific number of expected cases for its populations of interest (*exp_{ag & s}*) (Eq.5).

$$exp = \sum exp_{ag \& s} \quad (4)$$

Equation.4: The total for each department’s expected cases (*exp*) between 1992-2016 from previously calculate age-sex-specific expected cases (*exp_{ag & s}*) between 1992 and 2016¹.

The incidence rate and number of expected cases of CJD was recalculated for the whole of France at each set of maps as per this set of equations (Eq.1-3).

III) Livestock distribution in France and CJD

Distribution of livestock

Additional maps of the distribution of livestock in France were made (cattle, sheep, and pigs, Supp-Data.III). They were based on data from the Insee from the ministry of Agriculture for the year 2022². Each animal group had two maps done, coloured-in based on either the concentration of livestock heads per human being or per km².

Whether a department was considered to be livestock-dense was calculated using a single-department cluster analysis (cluster size = 1). Since only single centroids were considered, SaTScan and FlexScan were identical as they only differ at 2 or more centroids and thus SaTScan was chosen arbitrarily.

These were compared to the distribution of sCJD cases in the subgroup of people who were born and died within the same department (BandD) as these sCJD cases are less likely to have moved in their lifetimes and thus more appropriate to investigate a potential exposure to an exogenous risk factor.

Cows

When looking at the distribution of departments considered to have a significantly higher density of cattle than expected, we can see that cow rearing roughly excludes the southern Atlantic coast, the Mediterranean, Corsican and Alpine regions as well as Paris and its neighbouring departments. This leads to a large overlap of the departments appearing in any significant sCJD cluster in the BandD population and the departments with high cattle density that is significant when livestock is considered by either head per person (Fig.1.A) or head per km² (Fig.1.B).

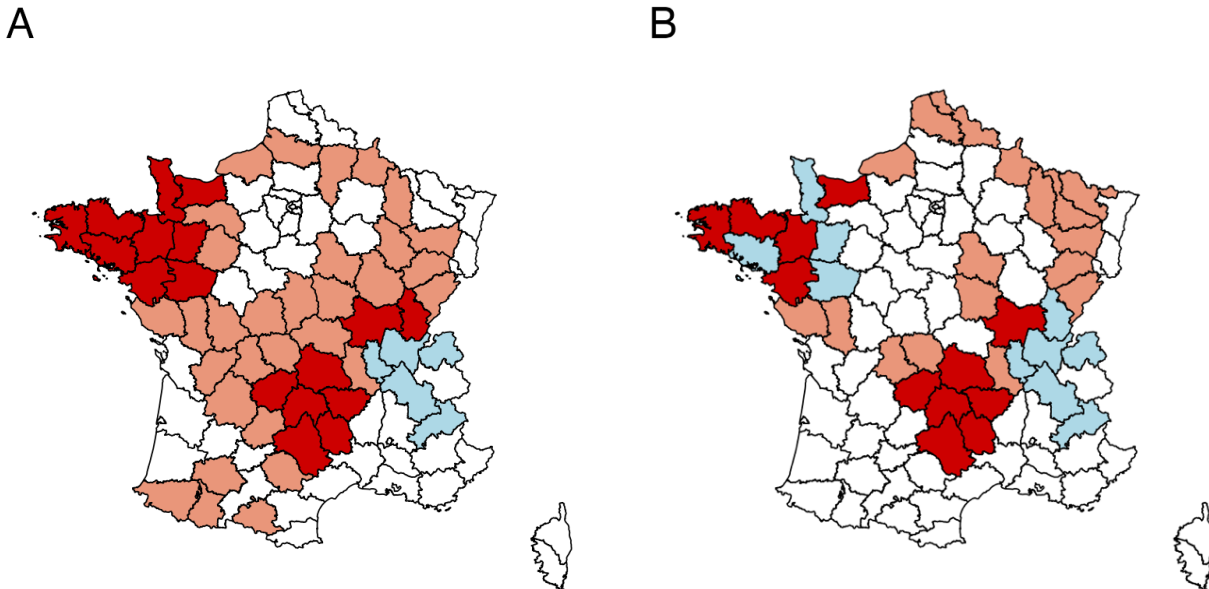
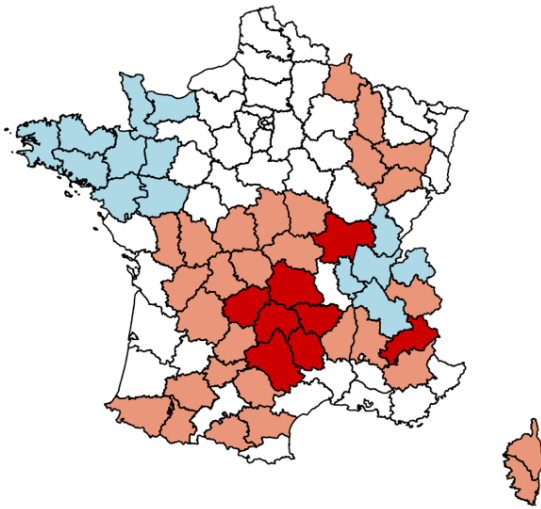


Figure.1: Maps of the French departments of cattle distribution (salmon coloured) determined through single department cluster analysis (SaTScan, cluster size = 1), compiled with all departments contained in at least one significant BandD sCJD cluster (all, M1 or V2 with both SaTScan or FlexScan) determined prior (blue) and their overlap (red). Correlation between livestock and sCJD was significant in the case of both **A**) livestock heads per human (p-value = 2.17×10^{-4} , χ^2 test) and **B**) livestock heads per km² (p-value = 1.82×10^{-5} , χ^2 test).

Sheep

When looking at the distribution of sheep husbandry-significant departments, it can be generally observed that the north of the country is excluded from significant sheep rearing as well as the coastal departments (Atlantic and Mediterranean). This leads to a non-significant overlap of the departments with high sheep density and BandD sCJD clusters (heads per person, Fig.2.A, and heads per km², Fig.2.B) as the Brittany sCJD cluster is completely free of significant sheep rearing departments.

A



B

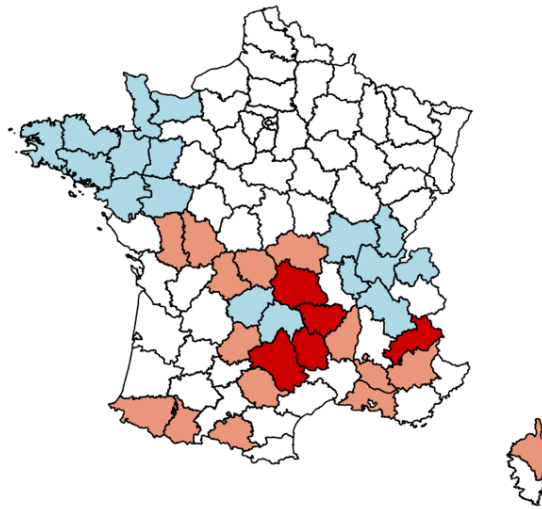
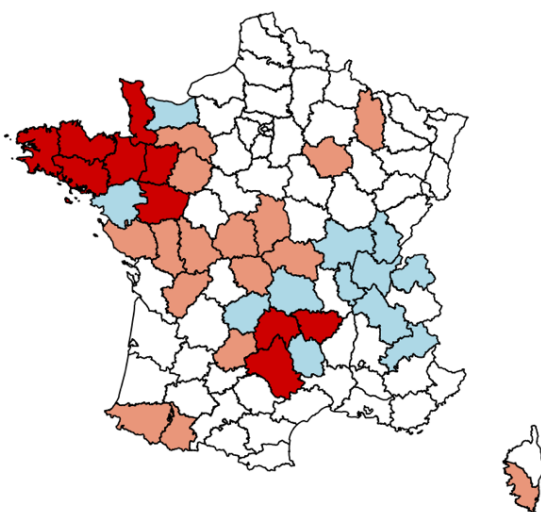


Figure.2: Maps of the French departments of sheep distribution (salmon coloured) determined through single department cluster analysis (SaTScan, cluster size = 1), compiled with all departments contained in at least one significant BandD sCJD cluster (all, M1 or V2 with both SaTScan or FlexScan) determined prior (blue) and their overlap (red). Correlation between livestock and sCJD was not significant in the case of either **A**) livestock heads per human (p-value = 0.76, χ^2 test) and **B**) livestock heads per km² (p-value = 0.49, χ^2 test).

Pigs

Finally, a third set of maps was drawn up, looking at the pig distribution in France. Here once more, a significant overlap was found. In terms of animals per person, the distribution of pig rearing is mostly located in the west, notably Brittany as well as a bit of central France (Fig.3.A). When it comes to the animals per km², it is interesting to see that even if the bulk of the BandD sCJD clusters does not overlap the pig-husbandry-rich regions (Fig.3.B), the entirety of the Brittany cluster (general SaTScan V2) overlaps pig-farming departments, resulting in a high significance of overlap between animal and sCJD.

A



B

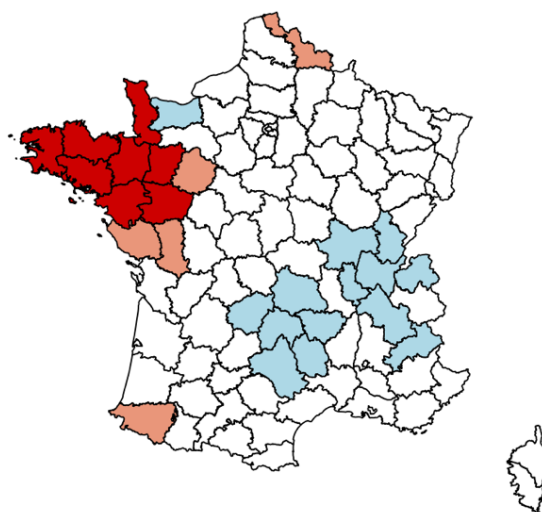


Figure.3: Maps of the French departments of porcine distribution (salmon coloured) determined through single department cluster analysis (SaTScan, cluster size = 1), compiled with BandD sCJD clusters determined prior (blue) and their overlap (red). Correlation between livestock and sCJD was significant in the case of both **A**) livestock heads per human (p-value = 0.01, χ^2 test) and **B**) livestock heads per km² (p-value = 4.33×10^{-4} , χ^2 test).

This comes with its own limitations: the livestock data extracted from the government of agriculture was the state of the livestock distribution in France in 2022. Of course, this year is not considered within the CJD data and thus is not ideal for this study. However, this was the most complete and best formatted national livestock data that could be found while conducting this project and it can be argued that whether an area in a country such as France can be considered prone to livestock rearing is not likely to change in half of a century.

REFERENCES

1. Insee. Estimation de la population au 1^{er} janvier 2023: Séries par région, département, sexe et âge de 1975 à 2023. <https://www.insee.fr/fr/statistiques/1893198#consulter>. January 24, 2023.
2. Insee, Ministère en charge de l'Agriculture A statistique agricole annuelle, données définitives. Cheptel présent dans les exploitations agricoles en fin d'année 2022 Comparaisons régionales et départementales. February 2024.