Vet. Res. 36 (2005) 615–628
© INRA, EDP Sciences, 2005
DOI: 10.1051/vetres:2005020

Original article

Poultry, pig and the risk of BSE following the feed ban in France – A spatial analysis

David ABRIAL[a]*, Didier CALAVAS[b], Nathalie JARRIGE[b], Christian DUCROT[a]

[a] Unité d'Épidémiologie Animale, INRA Theix, 63122 Saint-Genès-Champanelle, France
[b] Unité Épidémiologie, AFSSA Lyon, 31 avenue T. Garnier, 69364 Lyon Cedex 07, France

(Received 24 September 2004; accepted 16 December 2004)

Abstract – A spatial analysis was carried out in order to analyse the reason why the risk of Bovine Spongiform Encephalopathy (BSE) was spatially heterogeneous in France, during the period following the feed ban of Meat and Bone Meal to cattle. The hypothesis of cross-contamination between cattle feedstuff and monogastric feedstuff, which was strongly suggested from previous investigations, was assessed, with the assumption that the higher the pig or poultry density is in a given area, the higher the risk of cross-contamination and cattle infection might be. The data concerned the 467 BSE cases born in France after the ban of meat and bone meal (July 1990) and detected between July 1st, 2001 and December 31, 2003, when the surveillance system was optimal and not spatially biased. The disease mapping models were elaborated with the Bayesian graphical modelling methods and based on a Poisson distribution with spatial smoothing (hierarchical approach) and covariates. The parameters were estimated by a Markov Chain Monte Carlo simulation method. The main result was that the poultry density did not significantly influence the risk of BSE whereas the pig density was significantly associated with an increase in the risk of 2.4% per 10 000 pigs. The areas with a significant pig effect were located in regions with a high pig density as well as a high ratio of pigs to cattle. Despite the absence of a global effect of poultry density on the BSE risk, some areas had a significant poultry effect and the risk was better explained in some others when considering both pig and poultry densities. These findings were in agreement with the hypothesis of cross-contamination, which could take place at the feedstuff factory, during the shipment of food or on the farm. Further studies are needed to more precisely explore how the cross-contamination happened.

BSE / bovine / poultry / pig / spatial analysis

1. INTRODUCTION

In 1991, the first French case of Bovine Spongiform Encephalopathy (BSE) was described by Gouëllo [16]. Different control measures were introduced successively in France; the most important took place in July 1990, with the ban of meat and bone meal (MBM) for cattle, then in June 1996 with the removal of cadavers and specified risk material (SRM) from MBM used for animal feed, and in November 2000 with the total ban of MBM and certain types of tallow for animal feed. Despite these control measures, BSE cases were detected in animals born after 1990, called BAB cases (born after the ban), and after June 1996, called BASB cases (born after the

* Corresponding author: dabrial@clermont.inra.fr

second ban). Up to July 1st, 2004, 811 BAB and 83 BASB cases had been detected. In other countries, BSE cases born after the same type of control measures were also identified and the main source of infection has been hypothesised to be the cross-contamination between pig or poultry feed for which MBM were still authorised, and cattle feed[1] [10, 11, 37, 38]; this led to complementary control measures targeted on pig and poultry feed. However, the scientific evidence of such a link between the risk of BSE and pig and poultry feed has not been formally established so far, apart from a correlation study carried out by Wilesmith [36].

In previous studies (framework of the disease mapping) focused on the period with comprehensive surveillance of BSE in France, a spatial heterogeneity of the BSE risk has been highlighted in western France [1] and then on the whole French territory [2]. The main conclusion was that the risk of infection with the BSE agent is not randomly distributed. In order to analyse the reason why the BSE risk is spatially heterogeneous, a spatial analysis was carried out to study the hypothesis of cross-contamination between monogastric and cattle feedstuff as the source of infection for the BAB cases, which was strongly suggested by the investigations on the BSE cases[2]. The hypothesis is that a higher pig or poultry density in a given area results in a higher risk of cross-contamination of cattle feedstuff with monogastric feedstuff – either on the farm, at the factory or during the shipment – and thus an increased risk of exposure of cattle to the BSE agent. This is based on the fact that the French territory is covered with hundreds of feedstuff factories and the feed is used locally for the most part. The analysis presented in this article was done in the framework of geographical correlation studies [32], which have been applied in Human epidemiology since the nineteen-nineties [4, 17]. The goal was to examine geographical variations in exposure to environmental variables (risk factors) in relation to an epidemiological outcome measure (the number of BSE cases) on a geographical scale [14]. The method used to build and represent the model as well as to estimate the parameters involved three different techniques used together: the Hierarchical Bayesian approach [5, 23, 31], simulation with the Markov Chain Monte Carlo (MCMC) method [15] and Bayesian graphical modelling [34].

2. MATERIALS AND METHODS

2.1. Data

2.1.1. BSE cases

Epidemiological data on BSE were provided by the "Agence Française de Sécurité Sanitaire des Aliments" (AFSSA Lyon, France), in charge of the monitoring of BSE. The analysis was restricted to a time period of surveillance – between July 1st, 2001 and December 31, 2003 in order to get precise and comparable data on BSE incidence. During this period, the detection of BSE was based both on the Mandatory Reporting System of clinical suspicions and the comprehensive active surveillance programme based on rapid tests, carried out on every cattle aged 24 months and over, dead or slaughtered[3] [8]. These two systems were complementary since they

[1] DGAL, Information about BSE in France, [on line] (1997) http://www.agriculture.gouv.fr/esbinfo/esbinfo.htm [consulted 2 December 2004].

[2] Brigade Nationale d'Enquêtes Vétérinaires et Sanitaires. Enquête épidémiologique relative aux cas d'ESB survenus en France en 1999 [on line] (2000) http://www.agriculture.gouv.fr/esbinfo/pour_savoir_plus/enquetes&rapp/contenu/enquete_epide.pdf [consulted 2 December 2004].

[3] Calavas D., Ducrot C., L'ESB en France – Synthèse sur l'évolution de l'épizootie à partir des données disponibles au 1er janvier 2003. Agence Française de Sécurité Sanitaire des Aliments, [on line] (2003) http://www.afssa.fr/ftp/afssa/basedoc/RapportESB040203.pdf.pdf [consulted 2 December 2004].

allowed the screening of every cattle aged 24 months and over, dead or slaughtered. The BSE cases considered in the analysis were either clinically suspect animals confirmed at the national reference laboratory of AFSSA with Western blot or immunohistochemistry – i.e. cases found with the Mandatory Reporting System –, or test positive animals – using the rapid tests that passed European Union validation [29] – confirmed with the same two techniques, among the whole exiting cattle population captured within the active surveillance programme[3]. Among the BSE cases, only the BAB cases – born from January 1990 through June 1996 – were taken into account as the targeted population for studying the hypothesis of cross-contamination between pig or poultry feed and cattle feed.

2.1.2. Case location and geographical units

The geographical location of the BSE cases was defined by the centroid of the "commune" (the smallest French administrative unit analogous to a municipality) of the farm in which the case has been raised between the sixth and twelfth months after birth. All BSE cases located in the same geographical unit were pulled together. As explained in a previous work [2], the "cantons" (French administrative unit including five "communes" on average – France is divided into 3705 "cantons") are not homogenous in shape, size and neighbourhood. To overcome this problem, we used hexagons of 23 km width and 450 km^2 area as geographical units; the French metropolitan territory without Corsica was thus divided into 1240 hexagons, contiguous polygons labelled $i = 1, \ldots, 1240$. The size of the hexagon was constrained by both the geographical accuracy of the demographic data (hexagons must not be too little compared to the size of the "canton", a scale at which the demographic data were available), and the field knowledge about the average delivery area of a factory (hexagons must not be too large in order to properly describe the variations of the risk between factories).

The geographical data on the "cantons" perimeters and the "communes" centroids were provided by the GEOFLA® "France Métropolitaine" (IGN© Paris, version 6, 2002).

2.1.3. Background population

The background population was assessed by the demography of the female adult bovines, i.e. cows having calved, obtained from the Agricultural Census 2000 (CD-ROM edited by Agreste, 251 rue de Vaugirard, 75732 Paris, France). The background bovine population, as well as the covariates, were available at the level of the "canton". A spatial joint between the layers of cantons and those of hexagons was done with a Geographic Information System (ArcView GIS, ESRI Inc., Redlands, USA).

2.1.4. Covariates

The main goal was to evaluate the influence of the poultry and pig density on the BSE risk. In France, 286 million poultry and 14.7 million pigs have been registered in the Agricultural Census 2000. The number of poultry and pigs by geographical unit were considered as covariates, expressing these numbers per 100 000 for poultry (Pou_i) and per 10 000 for pigs (Pig_i), in order to simplify the numerical fitting of the models and the interpretation of the results.

2.2. Mapping method

The disease mapping models used are in the hierarchical Bayesian model framework [5, 23, 31]. The main interest of this method is the possibility to control the variability of the parameter of interest, the relative risk of BSE, by prior distribution. Consequently, the classical problem of overdispersion in disease mapping [25] can be overcome by smoothing the relative risk. Indeed, the assumption that the observed

number of cases follows a Poisson distribution is rarely verified with the observations mainly because of extreme values, which can be accounted for by smoothing. A way to smooth the risk is to use a spatial effect [27] because the geographical units are often not independent. Hierarchical models have rarely an explicit solution, and the parameters have to be estimated by simulation approaches such as the MCMC method. The models were built in three steps, first the basic disease mapping model using the MCMC method, secondly a strategy to choose a spatial effect and finally the incorporation of the covariates in the model. All models were based on the Bayesian graphical modelling [34]. This method uses the Directed Acyclic Graphs (DAG) constructed from a set of nodes (the stochastic variables of parameters and data) linked by edges representing the dependence between the nodes. The DAG (not shown in the article) permits to deduce, from prior information on the parameters and likelihood function of the data, the posterior distribution necessary to run the simulation with the MCMC method [15].

2.2.1. Basic model and adjustment

BSE is a non-contagious and rare disease, so it can be assumed that the observed numbers of BSE cases y_i in each of the 1240 hexagons follow a Poisson distribution.

$$y_i \sim P(\lambda_i)$$
$$\lambda_i = e_i.r_i \Leftrightarrow \ln(r_i) = \ln(\lambda_i) - \ln(e_i) \quad (1)$$
$$\text{with } i = 1, ..., 1240.$$

The parameter λ_i is the product of the expected number of BSE cases e_i on the basis of the overall French incidence and the Relative Risk (RR) of BSE r_i in a given hexagon. The expected number of BSE cases takes into account the demographic structure of the bovine population and it has been evidenced that BSE incidence varies according to the production type (dairy versus beef cattle) [13, 28, 37]. So, the population has been divided into two subpopulations:

$$e_i = p_{dairy}.DAIRY_i + p_{beef}.BEEF_i. \quad (2)$$

$DAIRY_i$ and $BEEF_i$ are the numbers of cattle in each hexagon given by the Agricultural Census 2000 and p_{dairy} and p_{beef} are the overall probabilities of infection assessed from the data. The RR r_i is the variation of the risk of BSE compared to a standard risk evaluated on the whole French territory.

2.2.2. Estimation of the parameters

Hierarchical models can be fitted by the MCMC method as implemented in WinBUGS, a free software for Bayesian inference using Gibbs Sampling (Medical Research Council, Biostatistics Unit of Cambridge, London, http://www.mrc-bsu.cam.ac.uk/bugs/welcome.shtml). Gibbs Sampling is an adaptation of the general Metropolis algorithm [15]. It consists in visiting each parameter (called node) in turn and simulating a new value for this parameter from its full conditional distribution, given the current values for the remaining parameters [26]. In the analysis, the 50 000 first cycles (burn-in of samples) of the Markov chain were discarded from the calculations. The effective and usable chain has a size of 100 000 cycles. The parameters of interest (RR and coefficient regression) were estimated with their posterior mean calculated from a sampling of 20 000 values of the usable chain (only 1 value every 5 cycles was used in order to reduce the autocorrelation). The stability of the chains was verified with the Heidelberger-Welch convergence diagnostic [18]. The tests of conformity about the parameters were made from the 95% prediction interval given by the quantile of the usable chain.

2.2.3. Spatial effects

The RR can be directly estimated by the standardised ratio y_i/e_i (maximum likelihood

estimation) if we assume that the RR are spatially independent. This, however, is not the case; therefore a spatial component needs to be added to the model. First, Besag et al. [6] developed a Conditional Autoregressive model (CAR) that was introduced in disease mapping by Clayton and Kaldor [9] as a spatial effect u_i based on a matrix of contiguities between geographical units. The prior distribution of this spatial effect is:

$$u_i \sim N(\bar{u}_{\partial i}, \tau_{ui}). \quad (3)$$

$\bar{u}_{\partial i}$ is the mean of the spatial components in the set ∂i of the hexagons adjacent to hexagon i (neighbouring) and τ_{ui} is the variance inversely weighted by the number of neighbours of hexagon i. This component is also called the "clustering effect" [33]. The CAR model is implemented in the geographical extension of WinBUGS version 1.4: GeoBUGS developed at the Department of Epidemiology and Public Health of the Imperial College at St Mary's Hospital, London.

Secondly, a spatial component h_i without spatial structure [6] can be added to the model. This component is a realisation of a Gaussian "white noise" of mean μ_{hi} and variance τ_h called "heterogeneity effect" [33]. The prior distribution of h is:

$$h_i \sim N(\mu_{hi}, \tau_h). \quad (4)$$

Both types of spatial effects added to the log of r_i were tested using three different models, one with a clustering effect alone (u_i), one with a "white noise" effect alone (h_i), and the last one with both.

2.2.4. Deviance Information Criterion

In order to compare models with variable complexity (a number of parameters and hierarchical levels), fitted with a MCMC method, Spiegelhalter et al. [35] developed the Deviance Information Criterion (DIC); it is a generalisation of the Akaike Information Criterion (AIC) that is not appropriate for the hierarchical models. DIC is calculated by adding the effective number of parameters (complexity) to the posterior mean deviance (adequacy) of a model. The effective number of parameters is assessed by the difference between the posterior mean of the deviance and the deviance at the posterior estimates of the parameters of interest. The "best fit" model is the one with the smallest DIC value. Before incorporating the covariates, the DIC was used to choose which spatial effect u_i and/or h_i was to be introduced. This criterion was assessed by WinBUGS at the same time as the MCMC simulation.

2.2.5. Incorporating the covariates in the model

The spatial model without a covariate was of the same kind as those already used in a previous work [2]. The covariates, Pou_i and Pig_i can be added linearly in the prior distribution of the logarithm of the RR r_i as explained by Lawson et al. [22].

$$\log(r_i) = spatial_i + b_0 + b_1.X_i. \quad (5)$$

In this equation, $spatial_i$ is the spatial effect u_i and/or h_i. The baseline risk b_0 is the average risk; we assumed that the prior distribution of this parameter is uniform. X_i represents a covariate and b_1 the regression coefficient. The covariates Pou_i and Pig_i were incorporated one at a time in the model and then together. The effect of each covariate on the RR was evaluated with a test of conformity at 0 on the regression coefficient H_0: $b_1 = 0$ and also with the DIC. The regression parameter b_1 can be interpreted as an odds ratio $\exp(b_1)$, indicating how much the RR (relative risk of BSE in a hexagon) is increased for each unit of the covariate. If the covariate significantly influences the RR (reject of H_0: $b_1 = 0$), b_1 can also be expressed as a Variation Rate (VR) of the RR [30], in percent of increase (or decrease) of the RR by unit of the covariate. The VR is calculated from equation (5)

Table I. Disease mapping models used to test the area effects. The variation of the Deviance Information Criterion (DIC) was calculated and compared to the DIC value of model 0.

Model	$\log(r_i) =$	DIC	Δ DIC ref. *model 0*
Model 0	b_0	1625.91	
Model 1	$b_0 + h_i$	1615.85	–10.1
Model 2	$b_0 + u_i$	1586.01	–39.9
Model 3	$b_0 + h_i + u_i$	1589.39	–36.5

which can be written as $r_i = e^{spatial_i}.e^{b_0}.(e^{b_1})^{X_i}$, then

$$VR = (\exp(\hat{b}_1) - 1).100\%. \quad (6)$$

Two types of prior distribution were used successively for the regression parameter. The first one was a Uniform prior $b \sim U(bnd_1, bnd_2)$. This prior assumed that the covariate (Poultry or pig) influences the RR in the same way in all the geographical units (only one parameter for 1240 hexagons). The hyperprior bnd_1 and bnd_2 are integers fixed after successive trials. With the second prior, we assumed that the covariate influences the RR differently from one hexagon to the other; in this case, the regression parameter was individualised and the prior was a CAR model as the spatial effect u_i,

$$\log(r_i) = spatial_i + b_0 + b_{1i}.X_i$$
$$b_{1i} \sim N(\bar{b}_{1\partial i}, \tau_{b1i}). \quad (7)$$

2.2.6. Residuals

The residuals were computed from the following equation [21],

$$\varepsilon_i = y_i - e_i\hat{r}_i. \quad (8)$$

The spatial location of the residuals of the model including the covariates was useful to evidence the geographical areas where the relative risk of BSE was not explained completely by the poultry and the pig density, and those with an overestimated risk compared to reality.

2.2.7. Hyperparameter

In the simulation, we assumed that the hyperparameter of variance τ_h, τ_{ui} and τ_{b1i} followed a Gamma prior with shape and scale parameter both equal to 0.01 as suggested by Browne [7].

3. RESULTS

In the period July 1st 2001 to December 31 2003, 550 BSE cases were detected in France, 4 born before the feed ban, 467 BAB and 72 BASB, the 7 left being secondary cases or having an unknown date of birth. Among the BAB cases, 380 were detected in the dairy population of 4.39 million cows (p_{dairy} = 1.405 cases per 100 000 cows) and 87 cases in the beef cattle population of 3.86 million cows (p_{beef} = 0.287 case per 100 000 cows).

3.1. Spatial effect

Table I shows the set of models with the spatial effects u_i (clustering effect) and h_i ("white noise"). The variation of the DIC indicates that the local spatial effect u_i alone gave the "best" model with a variation of –39.9. Model 3 with both h_i and u_i effects was worse than model 2 with u_i alone. Thus, we used the local spatial effect alone in further models.

For model 2, the RR was estimated and is shown in Figure 1 (map B). The RR ranged from 0.52 to 2.78. The test of conformity H_0: $r_i = 1$ was performed for each

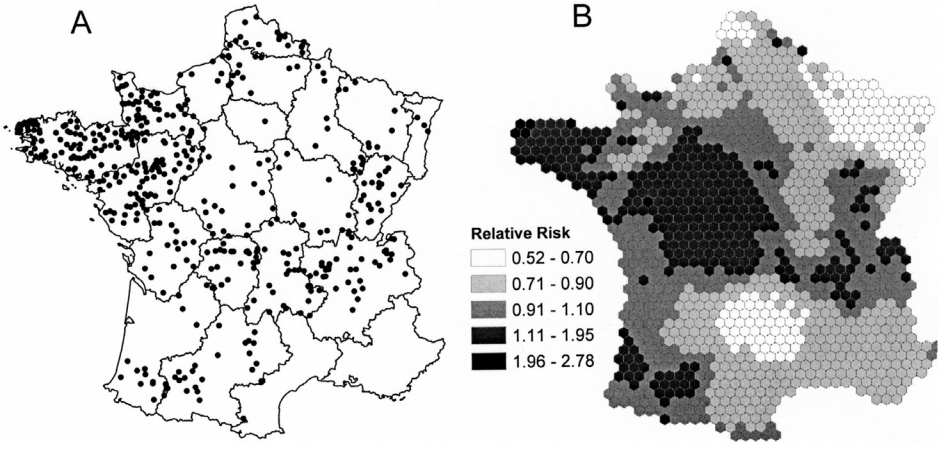

Figure 1. A – Location of the 467 BSE cases detected in France between July 1st 2001 and December 31 2003. The map is divided into 21 administrative regions. B – Relative risk of detection of BSE cases in France divided into 1240 hexagons. Model with a structured spatial effect (model 2), and MCMC method for estimation. The 303 hexagons with RR above 1.1 (the two darkest colors) had a significant test of conformity H_0:$r_i = 1$.

geographical unit and 303 hexagons (24%) had an RR signicantly above 1. In particular, four areas located in the west, south-west, centre and east of France (highlighted with the two darkest colours on the map) presented a significant RR.

3.2. Covariates

Table II illustrates the set of models carried out with the covariates poultry and/or pigs, each of them was used either as a common effect for all hexagons (models 4, 5, 6) or as a specific effect for each hexagon (models 7, 8, 9). From these results we deduced that the "best" models (lowest DIC value) are those with covariate pigs (model 5 with a unique regression parameter, and 9 with an individual regression parameter). The estimation of the regression parameters was associated with the 95% prediction intervals based on the 2.5% and 97.5% quantiles of the distribution of the parameter simulation (Markov Chain of 20 000 cycles). The prediction interval of the coefficient regression for poultry (b_1 in models 4 and 6) contained the value zero, so that the covariate poultry did not significantly influence the BSE risk. On the contrary, the covariate pigs significantly influenced the RR of BSE (b_1 in model 5 and b_2 in model 6), which confirmed the results deduced from the DIC values. The variation rate of the RR, calculated with equation (6), gave an estimated increase of the BSE risk of 2.4% per 10 000 pigs (model 5).

Figure 2 illustrates the geographical distribution of the poultry and pig density in France, based on the agricultural census of 2000. These demographic distributions are globally the same. The scenario with covariates poultry and pigs (model 7) was used to show the hexagons with a regression parameter significantly above zero. Two hexagons were found (mini-map A – Fig. 2) with a significant link between the RR of BSE and the density of poultry, and 31 hexagons (map B – Fig. 2) for pigs. In order to highlight the geographical areas where the density of poultry and pigs compared to those of cattle is important, we drew the map of the ratio of poultry to cattle (map C –

Table II. Estimate of regression parameters of the disease mapping model with covariates poultry and pigs. The variations of the Deviance Information Criterion (DIC) were calculated in comparison with the DIC value of model 2: DIC = 1586.01.The 95% prediction intervals were based on the quantile of the MCMC sample. The variation rate (VR) of the relative risk was calculated with equation (6).

Model	$\log(r_i) =$	Estimate	Prediction Interval	VR
			Models with a common effect for all hexagons	
Model 4	$b_0 + u_i + b_1.Pou_i$	$\hat{b}_0 = -0.189 \pm 0.09$	[–0.339, –0.056]	
	$\Delta DIC = -1.7$	$\hat{b}_1 = 0.001 \pm 0.0001$	[–0.005, 0.026]	not significant
Model 5	$b_0 + u_i + b_1.Pig_i$	$\hat{b}_0 = -0.194 \pm 0.08$	[–0.331, –0.065]	
	$\Delta DIC = -5.0$	$\hat{b}_1 = 0.024 \pm 0.0001$	[0.002, 0.045]	+2.4% per 10 000 pigs
Model 6	$b_0 + u_i + b_1.Pou_i + b_2.Pig_i$	$\hat{b}_0 = -0.219 \pm 0.08$	[–0.361, –0.086]	
	$\Delta DIC = -1.7$	$\hat{b}_1 = 0.005 \pm 0.0001$	[–0.012, 0.022]	not significant
		$\hat{b}_2 = 0.022 \pm 0.001$	[0.001, 0.045]	+2.2% per 10 000 pigs
			Models with one specific effect per hexagon	
Model 7	$b_0 + u_i + b_{1i}.Pou_i + b_{2i}.Pig_i$	$\hat{b}_0 = -0.155 \pm 0.08$	[–0.287, –0.033]	
	$\Delta DIC = -1.4$	$\hat{b}_{1i}$ from –0.052 to 0.062		$\hat{b}_{1i}$ significant in 2 hexagons
		$\hat{b}_{2i}$ from –0.031 to 0.063		$\hat{b}_{2i}$ significant in 31 hexagons
Model 8	$b_0 + u_i + b_{1i}.Pou_i$	$\hat{b}_0 = -0.191 \pm 0.08$	[–0.334, –0.057]	
	$\Delta DIC = -1.9$	$\hat{b}_{1i}$ from –0.047 to 0.058		$\hat{b}_{1i}$ significant in 1 hexagon
Model 9	$b_0 + u_i + b_{1i}.Pou_i$	$\hat{b}_0 = -0.165 \pm 0.08$	[–0.294, –0.44]	
	$\Delta DIC = -6.2$	$\hat{b}_{1i}$ from –0.024 to 0.057		$\hat{b}_{1i}$ significant in 32 hexagons

Fig. 2) and those of pigs to cattle (map D – Fig. 2). It was deduced that the regression parameter b_{1i} and b_{2i} were significant in hexagons with a high value for these ratios, the south west of France for poultry and the Bretagne region for pigs.

3.3. Residuals

Figure 3 is based on the residuals ε_i calculated for each hexagon from equation (8) for model 7, with covariates poultry and pigs (map A) and model 9 with covariate Pigs (map B). As explained previously, we used the residuals in order to evidence the hexagons where the BSE risk was not entirely explained by the covariates poultry and pigs. Indeed, if the residue ε_i is positive or higher than a threshold (set at 1 BSE case), the observed number of BSE cases y_i (observation of the BSE risk) is higher than the number resulting from the estimated RR (modelling of the BSE risk) by $e_i\hat{r}_i$. In this case, the true BSE risk is underestimated by the model with the covariates because all influencing factors have not been taken into account. On the contrary, if the residue is negative or lower than a threshold (set at –1 BSE case) there is an overestimation and the covariates predict too much risk.

The black hexagons (55 in map A – Fig. 3 and 64 in map B – Fig. 3) represent the geographical units where the BSE risk was underestimated by the model. The comparison of maps A and B shows 9 hexagons (highlighted with a black arrow in map B) that have an underestimated risk with the Pig covariate only and not with both pig and poultry covariates. This suggests that the poultry density might explain part of the risk in these hexagons.

The white hexagons (14 in map A – Fig. 3 and 20 in map B – Fig. 3) show areas where the model overestimated the BSE risk.

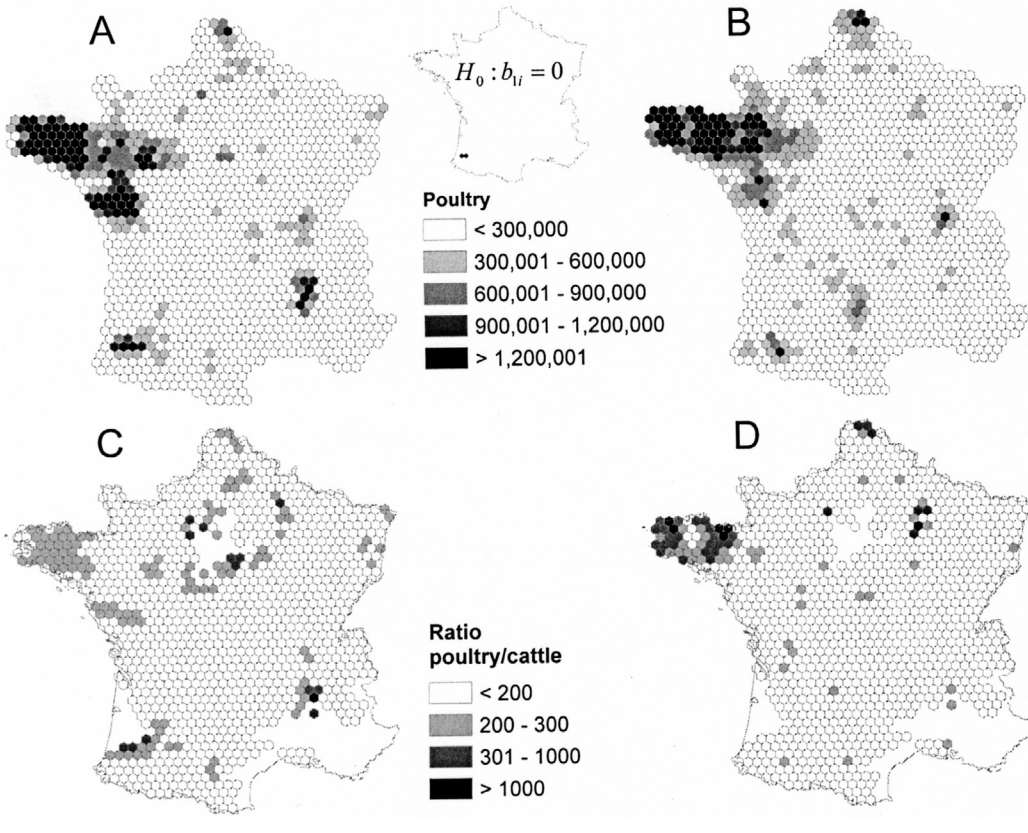

Figure 2. Geographical distribution of poultry density (map A), pig density (map B), ratio of poultry to catt pigs to cattle (map D). Data came from the agricultural census 2000. The maps show the 2 hexagons (map A) B) where the regression parameters b_{1i} and b_{2i} are significantly above 0 in model 7.

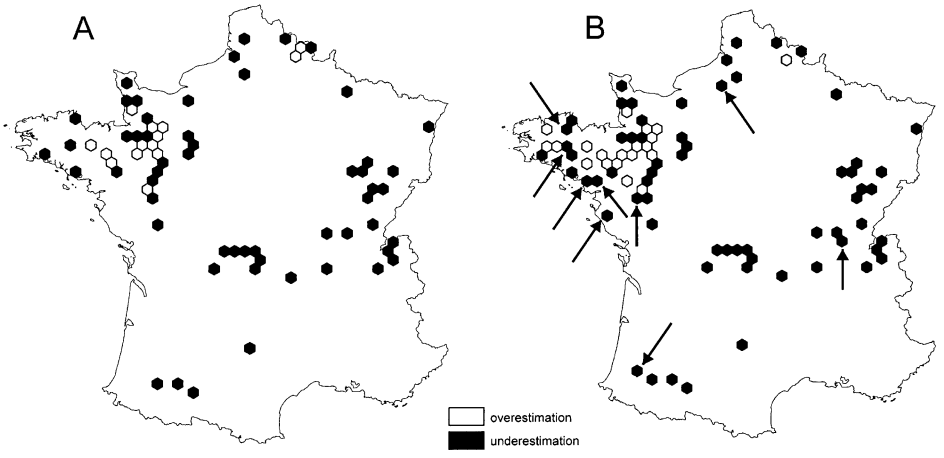

Figure 3. Residuals for model 7 (map A, pigs and poultry) and model 9 (map B, pigs only) calculated from equation (8). The black hexagons represent the geographical units with underestimated risk. The white hexagons represent those with overestimated risk. The 9 black arrows highlight the differences between the two maps. The threshold of 1 BSE case was used to compare the absolute values of the residuals.

4. DISCUSSION

The goal of this spatial analysis of the BSE risk in France was to assess the hypothesis of the infection of cattle by the cross-contamination between cattle feedstuff and monogastric feedstuff [12, 38], by means of disease mapping based on a Poisson model with covariates. The main problem of disease mapping is overdispersion. This extra Poisson variation was overcome in the analysis by the spatial prior added to the model in a hierarchical Bayesian approach [5]. The spatial effect with neighbourhood structure ("clustering effect") was sufficient to fully control the overdispersion, thus confirming previous work [2].

Coherent results were obtained in the analysis, since the best model based on the DIC included the only covariate that showed a significant effect, the pig density: model 5 (Tab. II) with a 5 points decrease of the DIC and model 9 (Tab. II) with a decrease of 9 points. This finding strengthened the choice of using the DIC to compare the models.

Another important point was the difference in the prior choice between the single regression parameters (models 4 to 6 – Tab. II) and the individual parameters, one for each hexagon (models 7 to 9 – Tab. II). A uniform (uninformative) prior was used for the single regression, while for the individual parameters an informative prior with a spatial structure was used. Firstly, it allowed a smoothing of the individual regression parameters, as for the "clustering effects", and secondly, the non-informative prior never produces stable Markov Chains with the set of data.

The methodological choice of aggregating the data per hexagon imposes a simplification of the geographical distribution of the cattle, poultry and pig population. Indeed, it needs to suppose that within each hexagon the three species are spatially homogenous and that all cattle were exposed to the infection risk in the same manner. This hypothesis seemed reasonable because one hexagon covers a small area (less than 500 km^2). Also, the results of spatial studies involving aggregates of

individuals as the unit of analysis, for example animals in specified geographical areas, are prone to possible bias [24], if a link found at the group level is not true at the individual level. Concerning BSE, the link observed at the aggregated level between pig/poultry density and BSE risk has a direct meaning at the individual level through the risk of cross-contamination of cattle feed, via different ways that are discussed later, and there is no reason to suspect any bias.

The main result is a global effect of the pig density, with a BSE risk increase of 2.4% per 10 000 pigs, and no global effect of the poultry density. An important point is that pig and poultry densities are spatially correlated in France (maps A and B – Fig. 2), and that the highest density areas for both species match the areas with an upper BSE risk. The fact that the results of the models including one single covariate (pigs or poultry) are in agreement with the models including both covariates tends to prove that the correlation between both covariates did not bias the results. This is the case for the models with a common parameter for all hexagons (models 4, 5, 6 on Tab. II), as well as for the models 7 to 9 (Tab. II) with individual regression parameters (one per hexagon). Indeed, only 1 or 2 hexagons had significant regression parameters for the poultry density, in the model with the covariate Poultry alone (model 8 – Tab. II) and also in the model with both covariates (model 7 – Tab. II). Inversely, models 7 and 9 (Tab. II) confirm that only the pig density influenced the BSE risk and not the poultry one.

In the hexagons where the individual regression parameters of models 7 to 9 (Tab. II) had a high value but were not significant, it can be assumed that this might be due to the low number of poultry or pigs in these hexagons. Indeed, according to the maps (Fig. 2) the individual regression parameters were significant only in hexagons with a high density of poultry and/or pigs. So a pig effect might also exist in the non significant zones that the model was maybe not able to detect.

Even if the global effect of the poultry density on the RR was not significant, the map of the residuals of model 7 that included both poultry and pig covariates (map B – Fig. 3) tended to show that the poultry density explained part of the BSE risk in 9 hexagons (highlighted by the black arrows). In these hexagons, the model with covariate Pigs alone underestimated the risk whereas those with both pigs and poultry did not. Also, in models 7 and 8, with individual parameters, few hexagons (1 and 2) showed a significant effect of the poultry density, located in the south-west of France (mini-map A – Fig. 2). It can be deduced from these findings that the effect of the poultry density on the risk of BSE cannot be completely dropped.

The black hexagons in map A (Fig. 3), even if they are quite few (55 hexagons – 4%), show the areas where the density of poultry and pigs considered together did not totally explain the BSE risk. This should be the case if other factors than those introduced in the model are involved in the BSE risk. However, another explanation might be that the model assumed an average effect of the pig density in the hexagons. We did not consider in this study that, with the same pig density in different areas, different risk factors characterising the feedstuff factories and the potential for cross-contamination on farms or at the factories might in fact produce different BSE risks. Furthermore, this would also be able to explain the question of those white hexagons with an overestimated risk of BSE (maps A and B – Fig. 3). If more stringent measures have been taken in certain areas compared to others, in order to control cross-contamination, the risk of BSE linked to the pig density might be less important in these areas than on average; so the model overestimates the risk of BSE. Most of the white hexagons were located in the Brittany region where it has been hypothesised that control measures were perhaps more stringent than

elsewhere during the period considered in the study [20].

The maps of the ratio of poultry to cattle population (map C – Fig. 2) and those of pigs to cattle (map D – Fig. 2) show that the areas with the highest ratio correspond to the areas with significant regression parameters (mini-maps – Fig. 2). Thus, both high density ratios of pig or poultry to cattle and significant regression parameters were observed in the south-west for poultry and in the west for pigs. This sounds coherent since it can be postulated that a higher ratio of pigs to cattle results in a higher risk of cross-contamination. In this situation, the production of a batch of food for cattle is assumed to follow those of a batch of food for pigs more often. This was also in agreement with Wilesmith's findings [36] of a positive correlation between the incidence of BSE in the UK and the ratio of pigs to cattle and poultry to cattle.

The hypothesis of cross-contamination with an ingredient used in poultry or pig feed has been considered since 1994 [10, 19] and is supported by the traces of meat and bone meal detected in cattle feed [3]. In the United Kingdom, Wilesmith et al. [36, 38] found that the response to the feed ban was less marked in the eastern region with a higher density of pigs and poultry, and reported that species-specific proteins were detected in feedstuffs using an ELISA. In their geographical clustering analysis of the BSE cases in Switzerland, Doherr et al. [11] evoked the possible cross-contamination of cattle feed with feedstuff produced for pigs and poultry in order to explain the presence of BSE clusters, both BSE clusters being in an area with high pig density, but they also stated that high densities of pigs and poultry were also present in other areas. These findings strongly support the hypothesis of cross-contamination, but no epidemiological analysis was formally done. The present study evidenced a statistical link between pig density and BSE risk in France for the period July 1st, 2001 to December 31, 2003, on animals born after the ban. However, no clear link was observed with poultry density, even if meat and bone meal have been used for poultry too, at a higher incorporation rate than for pigs[4].

Cross-contamination may occur at the factory, if food chains for monogastrics and ruminants are not clearly distinct, during the shipment of feed to the farms, or on the farms, especially on mixed farms with both cattle and pig or poultry operations. The fact that the link between poultry density and BSE risk is lower than between pig density and BSE risk might have several explanations. Among others, it could be (i) the consequence of differences in the proportion of cattle farms that have a pig operation compared to those with a poultry operation; (ii) the feasibility of feeding cattle with residual pig or poultry feedstuff, depending on factors such as differential appetence; (iii) the level of food chain separation in feedstuff factories between ruminant, pig and poultry food processing. At that stage, the mechanism underneath the observed link between density in monogastric species and BSE risk is not explained, and the respective role of pigs and poultry is not clearly established. In order to go further into the mechanism, which is important in a control perspective, further investigations are required. A case control study on BSE at the farm level is currently under analysis in France and should provide information on the respective risk factors of cross-contamination on the farms and at the factories. Furthermore, spatial analysis may provide a more in-depth understanding of the risk related to the feedstuff factories. It is important to investigate, apart from the real delivery area of the factory, the volume of feed processed for cattle, pigs and poultry respectively, the level of MBM incorporation in the different formulae, and the degree of differentiation of the process

[4] Sauvadet F., Vergnier M., Rapport de la commission d'enquête sur le recours aux farines animales, Document parlementaire de l'Assemblée Nationale, 3138 [on line] (2004) http://www.assemblee-nat.fr/rap-enq/r3138.asp [consulted 2 December 2004].

chains between feedstuff for ruminant and feedstuff for monogastric species. These different components of the potential risk should better explain the risk of cross-contamination at the factory.

ACKNOWLEDGEMENTS

The authors would like to thank the "Direction Générale de l'Alimentation" from the Ministry of Agriculture that provided the data of the surveillance programme of BSE, especially Daniel Lafon from the "Brigade Nationale d'Enquêtes Vétérinaires et Phytosanitaires", as well as Eric Morignat from the "Agence Française de Sécurité Sanitaire des Aliments" and Patrick Gasqui from the "Institut National de la Recherche Agronomique", for statistical advice. This study was funded in the framework of the "Groupement d'Intérêt Scientifique: Infections à Prions".

REFERENCES

[1] Abrial D., Calavas D., Lauvergne N., Morignat E., Ducrot C., Descriptive spatial analysis of BSE in western France, Vet. Res. 34 (2003) 1–11.

[2] Abrial D., Calavas D., Jarrige N., Ducrot C., Spatial heterogeneity of the risk of BSE in France following the ban of meat and bone meal in cattle feed, Prev. Vet. Med. 67 (2005) 69–82.

[3] AFSSA, Les risques sanitaires liés aux différents usages des farines et graisses d'origine animale et aux conditions de leurs traitements et de leur élimination, Opinion of the "Agence Française de Sécurité Sanitaire des Aliments", 2001.

[4] Alexander E.F., Cartwright R.A., McKinney P.A., Ricketts T.J., Leukaemia incidence, social class and estuaries: an ecological analysis, J. Public Health Med. 12 (1990) 109–117.

[5] Banerjee S., Carlin B.P., Gelfand A.E., Hierarchical modeling and analysis for spatial data, Chapman & Hall, London, 2004, pp. 99–128.

[6] Besag J., York J., Mollié A., Bayesian image restoration, with two applications in spatial statistics, Ann. Inst. Statist. Math. 43 (1991) 1–59.

[7] Browne W.J., Draper D., Goldstein H., Rasbash J., Bayesian and likelihood methods for fitting multilevel models with complex level-1 variation, Comput. Stat. Data An. 39 (2002) 203-225.

[8] Calavas D., Ducrot C., Baron T., Morignat E., Vinard J.L., Biacabe A.G., Madec J.Y., Bencsik A., Debeer S., Eliazsewicz M., Prevalence of BSE on western France by screening cattle at risk: preliminary results of a pilot study, Vet. Rec. 149 (2001) 55–56.

[9] Clayton D., Kaldor J., Empirical bayes estimates of age-standardized relative risks for use in disease Mapping, Biometrics 43 (1987) 671-681.

[10] Denny G.O., Hueston W.D., Epidemiology of bovine spongiform encephalopathy in Northern Ireland 1988 to 1995, Vet. Rec. 140 (1997) 302-306.

[11] Doherr M.G., Hett A.R., Rufenacht J., Zurbriggen A., Heim D., Geographical clustering of cases of bovine spongiform encephalopathy (BSE) born in Switzerland after the feed ban, Vet. Rec. 151 (2002) 467-472.

[12] Ducrot C., Calavas D., Epidémiologie de l'encéphalopathie spongiforme bovine et de la tremblante, in: Dodet B., Cathala F. (Eds.), Les maladies humaines et animales à prions, Elsevier, Paris, 2002, pp. 47-60.

[13] Ducrot C., Roy P., Morignat E., Baron T., Calavas D., How the surveillance system may bias the results of analytical epidemiological studies on BSE: prevalence rate among dairy versus suckler cattle breeds in France, Vet. Res. 34 (2003) 185-192.

[14] Elliott P., Wakefield J.C., Best N.G., Briggs D.J., Spatial epidemiology: methods and applications, in: Elliott P., Wakefield J.C., Best N.G., Briggs D.J. (Eds.), Spatial Epidemiology, Oxford, London, 2000, pp. 3-14.

[15] Gilks W.R., Richardson S., Spiegelhalter D.J., Markov chain Monte Carlo in Practice, Chapman & Hall, London, 1996.

[16] Gouëllo L., Analyse épidémiologique du premier cas d'encéphalopathie spongiforme bovine en France, Epidémiologie et Santé Animale 19 (1991) 63-70.

[17] Hatch M., Susser M., Background gamma radiation and childhood cancers within ten miles of a US nuclear plant, Int. J. Epidemiol. 19 (1990) 546-552.

[18] Heidelberger P., Welch P.D., Simulation run length control in the presence of an initial transient, Oper. Res. 31 (1983) 1109-1144.

[19] Hoinville L.J., Decline in the incidence of BSE in cattle born after the introduction of the "feed ban", Vet. Rec. 134 (1994) 247-275.

[20] La Bonnardière C., Calavas D., Abrial D., Morignat E., Ducrot C., Estimating the trend

of the French BSE epidemic over six birth cohorts through the analysis of abattoir screening in 2001 and 2002, Vet. Res. 35 (2004) 299-308.

[21] Lawson A.B., Browne W.J., Vidal Rodeiro C.L., Disease mapping with WinBUGS and MLwiN, Wiley, Aberdeen, 2003, pp. 17-28.

[22] Lawson A.B., Browne W.J., Vidal Rodeiro C.L., Disease mapping with WinBUGS and MLwiN, Wiley, Aberdeen, 2003, pp. 197-234.

[23] MacNab Y.C., Hierarchical Bayesian modeling of spatially correlated health service outcome and utilization rates, Biometrics 59 (2003) 305-316.

[24] Martin S.W., Meek A.H., Willeberg P., Veterinary Epidemiology – Principles and methods, Iowa State University Press, Ames, Iowa, 1987.

[25] Militino A.F., Ugarte M.D., Dean C.B., The use of mixture models for identifying high risks in disease mapping, Stat. Med. 20 (2001) 2035-2049.

[26] Mollié A., Bayesian and Empirical Bayes approaches to disease mapping, in: Lawson A., Biggeri A., Bohning D. (Eds.), Disease Mapping and Risk Assessment, Wiley, Aberdeen, 1999, pp. 15-29.

[27] Mollié, A., Richardson S., Empirical Bayes estimates of cancer mortality rates using spatial models, Stat. Med. 10 (1991) 95-112.

[28] Morignat E., Ducrot C., Roy P., Baron T., Vinard J.L., Biacabe A.G., Madec J.Y., Bencsik A., Debeer S., Eliazsewicz M., Calavas D., Targeted surveillance to assess of BSE in high risk populations in western France and the associated risk factors, Vet. Rec. 151 (2002) 73-77.

[29] Moynagh J., Schimmel H., Tests for BSE evaluated, Nature 400 (1999) 105.

[30] Pascutto C., Wakefield J.C., Best N.G., Richardson S., Bernardinelli L., Staines A., Elliott P., Statistical issues in the analysis of disease mapping data, Stat. Med. 19 (2000) 2493-2519.

[31] Richardson S., Best N., Bayesian hierarchical models in ecological studies of health-environment effects, Environmetrics 14 (2003) 129-147.

[32] Richardson S., Monfort C., Ecological correlation studies, in: Elliott P., Wakefield J.C., Best N.G., Briggs D.J. (Eds.), Spatial Epidemiology, Oxford, London, 2000, pp. 205-220.

[33] Richardson S., Monfort C., Green M., Draper G., Muirhead C., Spatial variation of natural radiation and childhood leukaemia incidence in Great Britain, Stat. Med. 14 (1995) 2487-2501.

[34] Spiegelhalter D.J., Bayesian graphical modelling: a case-study in monitoring health outcomes, J. R. Statist. Soc. C 47 (1998) 115-133.

[35] Spiegelhalter D.J., Best N.G., Carlin B.P., Van der Linder A., Bayesian measures of model complexity and fit, J. R. Statist. Soc. C 64 (2002) 583-639.

[36] Wilesmith J.W., Recent observations on the epidemiology of bovine spongiform encephalopathy, in: Gibbs C.J. (Ed.), Bovine Spongiform Encephalopathy. The BSE Dilemma, Springer Verlag, New York, 1996, pp. 45-55.

[37] Wilesmith J.W., Wells G.A.H., Cranwell M.P., Ryan J.B.M., Bovine spongiform encephalopathy: epidemiological studies, Vet. Rec. 123 (1988) 638-644.

[38] Wilesmith J.W., Ryan J.B.M., Stevenson M.A., Morris R.S., Pfeiffer D.U., Lin D., Jackson R., Sanson R.L., Temporal aspects of the epidemic of bovine spongiform encephalopathy in Great Britain: holding-associated risk factors for the disease, Vet. Rec. 147 (2000) 319-325.