Epidemiol. Infect. (2006), **134**, 712–718.
doi:10.1017/S095026880500542X Printed in the United Kingdom

Association between indicators of cattle density and incidence of paediatric haemolytic – uraemic syndrome (HUS) in children under 15 years of age in France between 1996 and 2001: an ecological study

R. HAUS-CHEYMOL*, E. ESPIE, D. CHE, V. VAILLANT, H. DE VALK AND J. C. DESENCLOS

Institut de Veille Sanitaire, Saint Maurice, France

(*Accepted 1 September 2005, first published online 22 December 2005*)

SUMMARY

Over the past years Shiga-like toxin-producing *Escherichia coli* (STEC) O157:H7 emerged as an important cause of severe gastrointestinal illnesses and haemolytic–uraemic syndrome (HUS) with up to 10% of children infected with STEC developing HUS. We conducted a geographical ecological study using the district as the statistical unit. For each district, we estimated the incidence of HUS among children <15 years for the period 1996–2001 from national HUS surveillance data and data obtained on cattle density. We used multivariate Poisson regression to quantify the relation, adjusted for covariates, between paediatric HUS incidence and exposure to cattle. In univariate analysis, a positive association was observed between several cattle-density indicators and HUS incidence. In multivariate analysis, HUS paediatric incidence was associated with dairy cattle density and the ratio of calves to children <15 years ($P<0{\cdot}001$). Our findings are consistent with previous studies in other countries and support the recommendation to limit exposure of children to dairy cattle and manure to reduce the risk of STEC infection.

INTRODUCTION

Over the past 20 years, Shiga-like toxin-producing *Escherichia coli* (STEC) O157:H7 have emerged as an important cause of severe gastrointestinal illness [1, 2]. The clinical spectrum of STEC infection includes diarrhoea, haemorrhagic colitis, haemolytic–uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura. HUS, defined as the association of haemolytic anaemia with fragmented red blood cells, thrombocytopenia and acute renal insufficiency, develops in up to 10% of patients infected with STEC [3]. It usually occurs in young children and is the major cause of acute renal failure in children in western countries [1].

The main reservoir for STEC is the intestine of healthy cattle, the carriage rate being greater in young animals than in older ones. Several reports indicate that other species such as sheep, deer and goat also harbour these organisms [4]. *E. coli* O157:H7 can survive in cattle faeces and the environment for up to 18 weeks [5]. Transmission to humans occurs mainly through the consumption of undercooked beef meat [1] or unpasteurized dairy products [6] and direct contact with contaminated animals or their faeces [7]. Person-to-person spread in family households or institutional settings [8, 9] and ingestion of vegetables and water contaminated by cattle faeces has also been implicated [10, 11].

In France, the majority of medical laboratories do not routinely examine stools for STEC, and STEC infections are not mandatory notifiable. Therefore,

* Author for correspondence: Dr R. Haus-Cheymol, Département d'Epidémiologie et de Santé Publique Nord, Ecole d'application du service de santé des armées, Ilot Bégin, Bat 18, 00498 Armees, France.
(Email: rachel.haus@wanadoo.fr)

since 1996, the surveillance of STEC infections has been based on a nationwide surveillance system for HUS in children <15 years old. Thirty paediatric nephrology units in public hospitals notify HUS cases on a voluntary basis to the National Institute of Public Health (Institut de Veille Sanitaire) [12]. Every year less than 100 children mostly <3 years old develop HUS with a case-fatality ratio of <1% [2]. Most cases are sporadic and the majority is caused by *E. coli* O157 infection.

Recently, two Canadian ecological studies identified an association between different indicators of livestock farming intensity and incidence of human STEC infections in Ontario [13, 14]. In France, in a national case-control study conducted during 2000–2001 [15, 16], personal contact with cattle was a significant risk factor for paediatric HUS but this was during the May–September period only (the period in which the highest incidence of *E. coli* O157 infections is observed in other countries) [13]. To explore further the risk of transmission from contact with cattle we conducted a geographical ecological study by analysing the association between district HUS incidence and cattle density.

MATERIALS AND METHODS

Study design

We used a geographical ecological study design in metropolitan France, the statistical unit being the district (there are 95 districts in metropolitan France).

Data sources

HUS district incidence

Data on HUS incidence were derived from the French national paediatric HUS surveillance system, a network of paediatric nephrology units of university-affiliated and general hospitals. The system covers all of metropolitan France and is thought to be representative of HUS occurrence in France [12]. In this system, a case is defined as any person aged <15 years in whom a clinical diagnosis of HUS (sudden onset of haemolytic anaemia with renal failure) withprodromal diarrhoea has been made by a paediatrician nephrologist on the basis of microangiopathic haemolytic anaemia (haemoglobin <10 g/100 ml and schizocytosis $\geqslant$2%) and renal failure (plasma creatinine >60 μmol/l if age <2 years, >70 μmol/l if age $\geqslant$2 years). Cases of HUS that occurred between 1996 and 2001 and that were reported to the national database were considered for inclusion in this study. Children who had travelled in the 15 days prior to onset, cases associated with *Shigella* or common source foodborne outbreaks were excluded. Secondary cases of an index case in a family or an institutional setting (e.g. nursery, day-care) were also excluded to limit the inclusion of paediatric HUS cases acquired by person-to-person transmission.

STEC infection was confirmed by the detection of antibodies against one or more of the 26 STEC serogroups (O1, O2, O4, O5, O9, O25, O26, O29, O55, O100, O103, O104, O105, O111, O112, O113, O115, O118, O127, O128, O136, O145, O153, O157, O163, O164).

The mean annual district incidence rate between 1996 and 2001 was standardized by age (<1, 2–4, 6–10, 11–15 years) by the direct method using the France metropolitan district of <15 years old distribution as reference, obtained from the Institut National des Statistiques et des Etudes Economique (INSEE).

Exposure variables

The cattle-density data were obtained from the annual census (1996–2001) of the French Agriculture Ministry (Ministère de l'Agriculture, de l'Alimentation, de la Pêche et des Affaires Rurales). Meteorological data were collected from the Meteo France network during the study period. Latitude and longitude of each district county's centroid were provided by the National Geographic Institute.

Exposure indicators

Prevalence and excretion of STEC depend on cattle age and type of cattle production [17, 18]. Consequently, five different variables of cattle density were defined: total cattle density (total cattle/total cultivated area); dairy cattle density (total dairy cattle >1 year old/total cultivated area); beef cattle density (total beef cattle >1 year old/total cultivated area); calf density (total calves <1 year old/total cultivated area); total adult cattle (total adult cattle >1 year old/total cultivated area).

Other indicators considered in the analysis included the ratio of calves to the population of children <15 years old and the proportion of cultivated land in a district ('rural degree' = total cultivated land/total surface). All cattle-density exposures were categorized into four classes according to quartile breaks.

https://doi.org/10.1017/S095026880500542X Published online by Cambridge University Press

Since the survival of STEC in the environment is influenced by climatic conditions [9], we also included weather indicators as continuous variables in the analysis: the median annual district temperature and the median annual district precipitation. The geographical coordinates of each district county's centroid (latitude, longitude) were also included in the analysis to take into account a potential geographical gradient.

Statistical analysis

To study the relation between district incidence rate of paediatric HUS and cattle-density indicators we used the multiple Poisson regression model [19]. To compute expected paediatric HUS cases for each district, national rates for the whole population of children <15 years old for the same period were applied to the person-years of each district using a model equation with external standard rates. The standardized incidence ratio (SIR), i.e. the ratio of the observed to the expected number of incident cases was used to estimate the risk of HUS for the different exposure categories.

Univariate associations between each variable and the district incidence rate of paediatric HUS were examined by calculating univariate Poisson regression relative risk (RR) and their 95% confidence intervals (CIs). Each cattle-density variable associated with paediatric HUS in univariate analysis with $P<0{\cdot}2$ was introduced in a multivariate Poisson regression model with other variables that were associated with HUS ($P<0{\cdot}2$). Latitude and longitude were both included in the multiple Poisson regression even if not statistically significant at the 0·2 level in order to maintain a symmetrical expression of the coordinate system [20]. Interaction effects were considered between longitude and latitude. The analysis was performed using Egret software [21].

RESULTS

Number and characteristics of cases

A total of 451 cases of paediatric HUS that occurred during the period 1996–2001 were included in the study with a mean annual rate of 0·6 cases/100 000 children aged <15 years. The number of cases by district varied from 0 to 45 cases (median 5 cases); no cases were recorded in 12 districts (Fig. 1). About half of the cases (47·2%) had occurred during the summer months (June–September). Diarrhoea was bloody in 253 (59·9%) of the 422 patients for whom the information was available. Three (0·7%) of the 451 cases died. In total, 391 (86·7%) of the 451 patients were tested serologically. Antibodies against one or more of the 26 serogroups tested were found in 221 (56·5%) of these 391 patients. Antibodies against serogroup O157 [alone or in association with antibodies against (an)other serogroup(s)] were found in 204 cases (92·3% of cases who tested positive).

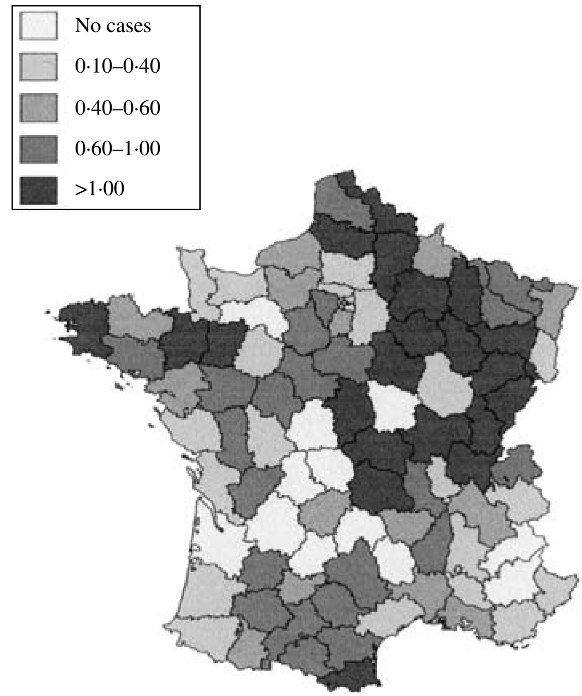

Fig. 1. Yearly incidence of paediatric haemolytic–uraemic syndrome (per 100 000 population), France, 1996–2001.

Univariate analysis

In univariate analysis, all variables but longitude were associated ($P<0{\cdot}05$) with paediatric HUS and the unadjusted RR for the total cattle density, the calves $\leqslant 1$ year old density and the cattle >1 year old density increased for each additional level of exposure (Tables 1 and 2).

Multivariate analysis

Because all cattle-density variables were very much collinear ($r>0{\cdot}80$, data not shown), we considered three distinct models of cattle-density variables adjusted for other variables: (1) the total cattle-density model; (2) the beef cattle-density and dairy

Table 1. *Incidence of paediatric HUS in children <15 years of age [relative risk (RR) with 95% confidence intervals (CI) obtained from univariate regression model], France 1996–2001*

Variables	Incidence rate (per 100 000/year)	RR	95% CI	*P*
Ratio calves/children <15 years[a]				<0·001[b]
0–0·12	0·52	1		
0·12–0·46	0·72	1·41	1·11–1·78	0·004
0·46–1·20	0·92	1·80	1·42–2·30	<0·001
1·20–7·45	0·67	1·32	0·97–1·8	0·08
Cattle density[a]				<0·001[b]
0–0·20	0·49	1		
0·20–0·5	0·59	1·21	0·91–1·64	0·22
0·5–0·9	0·76	1·54	1·21–1·98	<0·001
0·9–1·63	0·86	1·75	1·35–2·25	<0·001
Calves ⩽1 year density[a]				<0·001[b]
0–0·04	0·50	1		
0·04–0·11	0·58	1·18	0·88–1·59	0·26
0·11–0·22	0·78	1·56	1·22–1·98	<0·001
0·22–0·46	0·84	1·69	1·31–2·20	<0·001
Cattle >1 year density[a]				<0·001[b]
0–0·14	0·49	1		
0·14–0·39	0·60	1·23	0·92–1·66	0·18
0·39–0·70	0·64	1·31	1·00–1·70	0·05
0·70–1·24	0·95	1·95	1·53–2·47	<0·001
Dairy cattle density[a]				<0·001[b]
0–0·04	0·44	1		
0·04–0·11	0·68	1·57	1·16–2·14	0·003
0·11–0·29	0·64	1·48	1·11–1·97	0·007
0·29–0·90	0·91	2·09	1·64–2·67	<0·001
Beef cattle density[a]				<0·001[b]
0–0·08	0·49	1		
0·08–0·2	0·63	1·31	0·99–1·72	0·05
0·2–0·33	0·97	1·99	1·56–2·53	<0·001
0·33–0·84	0·64	1·32	1·00–1·75	0·048

[a] Categorized variable.
[b] Global Wald statistics.

cattle-density model and (3) the ratio of calves to the population of children <15 years old model. All saturated models included the rural degree and weather variables as well as latitude and longitude. In the first final model, cattle density was not associated with paediatric HUS incidence rate ($P=0{\cdot}21$), but the rural degree was (RR 1·07, 95% CI 1·01–1·15). In the second model only dairy cattle density (Fig. 2) remained associated with paediatric HUS incidence (Table 3). The third model indicated a significant association between the ratio of calves to the population of children <15 years old and paediatric HUS incidence ($P<0{\cdot}001$, Table 4). However, we note that the CIs of the coefficients (RR) for all densities greatly overlap.

DISCUSSION

In this geographical ecological study, we have found a significant positive association between several cattle-density variables and paediatric HUS in a univariate analysis. Two cattle-density variables remained significant in the multivariate analysis but we note that the CIs of the coefficients (RR) for all densities greatly overlap.

Table 2. *Incidence of paediatric HUS in children <15 years of age [relative risk (RR) with 95% confidence intervals (CI) obtained from univariate regression model], France 1996–2001*

Variables	RR	95% CI	P
Rural degree[ab]	1·14	1·09–1·20	<0·001
Latitude[a]	1·11	1·06–1·16	<0·001
Longitude[a]	0·97	0·93–1·00	0·08
Climatic temperature[a]	0·91	0·85–0·95	<0·001
Precipitation[ac]	1·07	1·01–1·12	0·01

[a] Continuous variable.
[b] RR for 10% rural degree increase.
[c] RR for 100 mm precipitation per year increase.

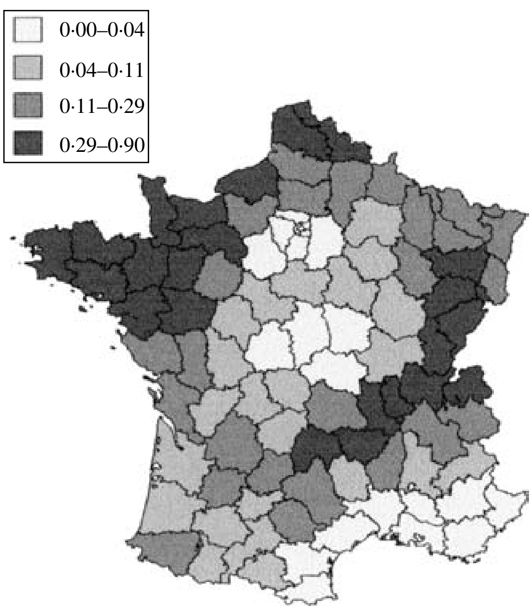

Fig. 2. Yearly dairy cattle density per hectare of cultivated area (total dairy cattle/total cultivated area), France, 1996–2001.

Table 3. *Multivariate Poisson regression production type model (dairy or beef cattle), France 1996–2001*

Variables	RR (95% CI)	P
Dairy cattle density[a]		<0·001[d]
0–0·04	1	
0·04–0·11	1·69 (1·23–2·35)	0·001
0·11–0·29	1·39 (1·04–1·85)	0·02
0·29–0·46	1·85 (1·44–2·38)	<0·001
Latitude[bc]	1·08 (1·03–1·14)	<0·001
Longitude[bc]	1·00 (0·96–1·04)	0·90

RR, Relative risk; CI, confidence interval.
[a] Categorized variable.
[b] Continuous variable.
[c] No significant interaction between latitude and longitude.
[d] Global Wald statistic.

Table 4. *Multivariate Poisson regression for ratio of calves cattle to children <15 years old population model, France 1996–2001*

Variables	RR (95% CI)	P
Ratio of calves to children[a]		<0·001[d]
0–0·12	1	
0·12–0·46	1·30 (1·03–1·65)	0·03
0·46–1·20	1·91 (1·48–2·48)	<0·001
1·20–7·45	1·33 (0·96–1·83)	0·08
Latitude[bc]	1·12 (1·07–1·18)	<0·001
Longitude[bc]	1·01 (0·97–1·05)	0·54

RR, Relative risk; CI, confidence interval.
[a] Categorized variable.
[b] Continuous variable.
[c] No significant interaction between latitude and longitude.
[d] Global Wald statistic.

Ecological studies, in which the unit of analysis is a population or a group of persons, offer many advantages [22–24]: they are less expensive and take less time than studies in which the data are gathered for each individual included. Even a small increase in risk can be detected in ecological studies because it is often feasible to study large populations. However, ecological studies have limitations, the 'ecological bias' or 'ecological fallacy' being the major one. The ecological fallacy results from making a causal inference about individual phenomena on the basis of observations of groups [23]. In ecological studies, the use of aggregated or grouped rather than individual data makes it difficult to establish the individual link between the exposure and the outcome [23]. This bias results from two components: (1) aggregation bias – due to the grouping of individuals; and (2) specification bias – due to the confounding effect of the 'group' itself [23]. Another limitation of ecological studies is the difficulty of taking into account confounding factors in the analysis. The introduction of a geographical gradient in a multiple Poisson regression model reduces strong spatial correlation and can also be considered as a proxy of other confounding variables [17]. In our study we did not take the migration bias into account [22]. Migration bias refers to the fact that migration within, into or out of each study population group is related to the factor of interest and such migration distorts the assessment

https://doi.org/10.1017/S095026880500542X Published online by Cambridge University Press

of the relationship between the exposure and outcome of interest [22]. This bias may affect the validity of ecological studies for long latency disease which does not apply for paediatric HUS for which the incubation period is around 1 week. Another problem with ecological analysis is that some of the exposure variables (i.e. the cattle-density indicators) are so highly correlated that it becomes difficult or impossible to distinguish their individual influences on the outcome variable which can interfere with the interpretation of multiple regression results. Multicollinearity is most problematic for ecological studies involving geographically defined units of analysis that are large and/or few in number [23], which is to some extent the case in our study. Because the number of paediatric HUS cases is limited we could not use a smaller level of geographical analysis such as the county or the zip code, as done previously in a study of legionellosis in France [24].

Poisson regression is now used routinely for ecological analysis (particularly in cancer epidemiology) [17, 20, 24], but for infectious diseases, this method is often inappropriate because of the non-independence of events due to person-to-person transmission [17]. In this study, we have reduced this potential problem by excluding paediatric HUS cases related to person-to-person transmission.

Recently, two Canadian ecological studies tested several livestock density indicators and demonstrated a spatial association between the incidence of human STEC infection and cattle density [13, 14]. One study showed that the highest r^2 alue model was the one that included the ratio of adult beef cattle to human population, while we found only dairy cattle to be associated with paediatric HUS. We also found a signifiant association between the ratio of calves to children <15 years old population and paediatric HUS incidence, although the RR did not increase linearly with increased exposure (Table 4). The differences found in other studies between production group cattle (adult dairy or adult beef) may reflect differences in study design and the way data on cattle are collected as well as differences in STEC excretion rates. Comparisons between STEC excretion rates in international studies are difficult because North American and European cow-calves production differs greatly. Consequently, the cattle prevalence data published in a North American country cannot be compared to European data without explanation [18]. In our study, the association between adult dairy cattle density and paediatric HUS incidence may be attributed to dairy cattle industry management practices (increased size of the dairy industry, manure practices, etc.); or differences in the diet of dairy and beef cattle. Acid resistance of some STEC may influence their transmission from cattle to humans and a recent study suggests than cattle fed mostly grain had a lower colonic pH value and more acid-resistant STEC that cattle fed only hay [25].

In this study, we also introduced different weather indicators (median annual district temperature and median annual district rainfall) because these variables may influence the transmission of STEC as suggested by the seasonal fluctuation of HUS. In 2000 in Ontario floods were believed to have contaminated a water supply with manure and STEC [9]. However, our results failed to show any association for these two variables and HUS incidence in the multivariate analysis.

In conclusion, the results of our geographical ecological study suggest that paediatric HUS incidence is associated with dairy cattle and calves density. These findings are consistent with several other studies in other countries and confirm the interest of limiting the exposure of children to cattle and their manure to reduce the occurrence of paediatric HUS in France.

ACKNOWLEDGEMENTS

The authors thank the departments of paediatric nephrology of the university and general hospitals that contributed human data to the HUS network. Thanks are also due to the French Meteorological Network and the documentation centre of the French Agriculture Ministry. This work was supported by the Fondation pour la Recherche Médicale, France.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Bell BP, Goldoft M, Griffin PM, et al.** A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. J Am Med Assoc 1994; **272**: 1349–1353.
2. **Haeghebaert S, Vaillant V, Espie E, Bouvet P, Grimont F, et le Réseau des néphrologues pédiatres.** Paediatric haemolytic uraemic syndrome (HUS) in children under 15 years of age in France in 2001 [in French]. BEH 2003; **20**: 89–91.

3. **Griffin PM, Tauxe RV.** The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *Escherichia coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991; **13**: 60–98.
4. **Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F.** Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J Clin Microbiol 1993; **31**: 2483–2488.
5. **Fukushima H, Hoshina K, Gomyoda M.** Long-term survival of shiga toxin-producing *Escherichia coli* O26, O111, and O157 in cattle feces. Appl Environ Microbiol 1999; **65**: 5177–5181.
6. **Chapman PA, Wright DJ, Higgins R.** Untreated milk as a source of verotoxigenic *Escherichia coli* O157 [Letter] [published Erratum appears in Vet Rec 1993; 133: 252]. Vet Rec 1993; **133**: 171–172.
7. **Locking ME, O'Brien SJ, Reilly WJ, et al.** Risk factors for sporadic cases of *Escherichia coli* O157 infection: the importance of contact with animal excreta. Epidemiol Infect 2001; **127**: 215–220.
8. **Crump JA, Sulka AC, Langer AJ, et al.** An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med 2002; **347**: 555–560.
9. **Renwick SA, Wilson JB, Clarke RC, et al.** Evidence of direct transmission of *Escherichia coli* O157:H7 infection between calves and a human. J Infect Dis 1993; **168**: 792–793.
10. **Michino H, Araki K, Minami S, et al.** Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. Am J Epidemiol 1999; **150**: 787–796.
11. **Krewski D, Balbus J, Butler-Jones D, et al.** Managing health risks from drinking water – a report to the Walkerton inquiry. J Toxicol Environ Health A 2002; **65**: 1635–1823.
12. **Decludt B.** Paediatric haemolytic uraemic syndrome (HUS) in children under 15 years of age in France. Epidemiology and related agents [in French]. Saint Maurice: Institut de Veille Sanitaire, 1997.
13. **Michel P, Wilson JB, Martin SW, Clarke RC, McEwen SA, Gyles CL.** Temporal and geographical distributions of reported cases of *Escherichia coli* O157:H7 infection in Ontario. Epidemiol Infect 1999; **122**: 193–200.
14. **Valcour JE, Michel P, McEwen SA, Wilson JB.** Associations between indicators of livestock farming intensity and incidence of human Shiga toxin-producing *Escherichia coli* infection. Emerg Infect Dis 2002; **8**: 252–257.
15. **Haeghebaert S, De Parscau L, Le Fur J, et al.** Cluster of paediatric haemolytic uraemic syndrome (HUS) in children under 15 years of age in Britanny, France, 1993–2000 [in French]. BEH 2001; **37**: 181–183.
16. **Vaillant V, Espié E.** Risk factors for paediatric haemolytic uraemic syndrome (HUS) in children under 15 years of age in France. Control case study 2000–2001 [in French]. Saint Maurice: Institut de Veille Sanitaire, 2003.
17. **Meyer-Broseta S, Bastian SN, Arne PD, Cerf O, Sanaa M.** Review of epidemiological surveys on the prevalence of contamination of healthy cattle with *Escherichia coli* serogroup O157:H7. Int J Hyg Environ Health 2001; **203**: 347–361.
18. **Hancock DD, Besser TE, Rice DH, Ebel ED, Herriott DE, Carpenter LV.** Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. Prev Vet Med 1998; **35**: 11–19.
19. **Viel JF.** Poisson regression in epidemiology [in French]. Rev Epidemiol Sante Publique 1994; **42**: 79–87.
20. **Viel JF.** Radon exposure and leukaemia in adulthood. Int J Epidemiol 1993; **22**: 627–631.
21. **Egret.** Software. Seattle: Egret, Statistics and Epidemiology Research Corporation, 1990.
22. **Tong S.** Migration bias in ecologic studies. Eur J Epidemiol 2000; **16**: 365–369.
23. **Morgenstern H.** Uses of ecologic analysis in epidemiologic research. Am J Public Health 1982; **72**: 1336–1344.
24. **Che D, Decludt B, Campese C, Desenclos JC.** Sporadic cases of community acquired legionnaires' disease: an ecological study to identify new sources of contamination. J Epidemiol Comm Health 2003; **57**: 466–469.
25. **Diez-Gonzalez F, Callaway TR, Kizoulis MG, Russell JB.** Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science 1998; **281**: 1666–1668.