Abstract. Concern is growing in Europe about alveolar echinococcosis (AE) with the increase in grassland rodent and red fox populations, intermediate and definitive hosts for *Echinococcus multilocularis*, respectively. The objective of this study was to assess the influence of rodent densities on human AE distribution. Spatial Poisson regression analyses were performed with geomorphologic features, landscape composition, climatic characteristics, and water vole density as independent variables. The outcome consisted of AE cases diagnosed over the period 1980–1992. High vole density yielded a 10-fold risk (relative risk [RR] = 10.34, 95% confidence interval [CI] = 2.78–38.39), and the first plateau (400–700 m altitude) compared with the plain (200–400 m) was associated with a large increase in risk (RR = 7.10, 95% CI = 1.30–38.63). These results confirm that human AE is strongly influenced by the densities of arvicolid species. Foxes feeding almost exclusively on grassland rodents when the latter expand could mediate this relation.

Alveolar echinococcosis (AE) is a very rare and severe zoonosis due to the hepatic development of the larval cestode *Echinococcus multilocularis*. The latter is largely restricted to wild animals, involving rodent intermediate hosts, and primarily red foxes as definitive hosts. Humans can become exposed after ingesting tapeworm eggs through direct contact with definitive hosts, or by food contaminated by feces of these infected carnivores.\(^1\)

The human disease risk appears relatively low within the distribution range of the parasite, but several hotspots have been observed in North America, Euro-Siberia, and Asia. In eastern France, such an area of high endemicity exists. For the period 1971–1989, 85 cases were identified in the Franche-Comté region, corresponding to an annual diagnostic incidence rate of 0.4 per 100,000 inhabitants.\(^1\) This estimate is much lower than that observed in St. Laurent Island, Alaska (annual incidence rate of 65 per 100,000), or in Gansu Province in China, one of the most highly endemic areas for AE.\(^2\) Nevertheless, concern is growing in this area (just as in all of Europe), especially with the increase in grassland rodent and red fox populations.

Little is known about the transmission patterns and processes involved in this zoonotic disease, which is cycled sylvatically rather than synanthropically. Several characteristics of the *E. multilocularis* life cycle represent many obstacles to transmission studies. This parasite affects a low proportion (< 1%) and is overdispersed between rodent intermediate host species. More than 40 species of rodents can serve as intermediate hosts, among which 6 have been found to be infected in France: common vole (*Microtus arvalis*), earth vole (*Microtus subterraneus*), red-backed vole (*Clethrionomys glareolus*), fossorial water vole (*Arvicola terrestris scherman*), muskrat (*Ondatra zibethicus*), and house mouse (*Mus musculus*).\(^1\) Moreover, population structure of wild intermediate and definitive hosts is highly variable both in time and space, and is hardly accessible to investigations. Finally, the human latency period, corresponding to the development of the larval stage, is long (5–20 years), impeding follow-up studies if migration occurs. As a result, one cannot extrapolate findings dealing with short time span or small spatial scales for animal hosts, or easily identify individual occupational or recreational risk factors for humans. Therefore, transmission patterns should be investigated on the basis of ecologic exposure variables relevant to large scales and wide time spans.

Such an approach has been recently carried out in the light of an ecologic theory linking landscape composition and interannual variations of small mammals densities, namely, the Ratio of Optimal to Marginal Patch Area (ROMPA) hypothesis. According to this hypothesis, population dynamics of small mammals would depend on the ratio of their optimal habitat in a landscape (e.g., grassland for *A. terrestris*). At a low ratio, the landscape matrix (ploughed fields, forest, permanent grassland, uncultivated land plus areas without forests) serves as a large dispersal sink in which young animals cannot disperse successfully and the population densities remain constantly low in the optimal habitats. At a high ROMPA, production of young cannot be compensated by unsuccessful dispersion and rodent density reaches a very high level.\(^1,6\) Landscape composition would therefore drive both the population dynamics of arvicolid species and the host-parasite interaction (foxes feed almost exclusively on grassland arvicolid species, with their diet becoming more diversified only during decreases in rodent populations).\(^7,8\)

The prevalence rates of *E. multilocularis* in foxes are remarkably high (20–70%) in endemic areas. In addition, fox populations have grown in Europe at least since the 1960s, and more so since the introduction of immunization against rabies in the 1980s.\(^9\) Furthermore, prevalence rates in foxes are getting higher in southern Germany since the 1950s,\(^10\) and the parasite distribution range seems to expand to areas where it was not previously recorded, such as eastern Europe (Poland).\(^11\) Consequently, concern is growing in Europe about AE, which could represent an emerging disease. Thus, increasing the understanding of this larval cestode transmission is urgently needed.

Our objective was to assess whether the distribution of human AE was linked to rodent species densities, while accounting for landscape composition. Since land composition and small mammal communities are spatially defined, a natural design of analysis appeared to lie within the framework of ecologic regression analyses.
The Doubs Département (part of the Franche-Comté region), a mainly rural area with a stable population, and a region of large-scale water vole outbreaks from the beginning of the 1970s, represented a unique opportunity to provide insight into the associations between landscape composition, rodent densities, and occurrence of human AE.

MATERIALS AND METHODS

Study site. The Doubs Département (485,000 inhabitants) is located in eastern France at altitudes between 200 and 1,450 m, and is characterized by the following geomorphological features in a northeast to southwest direction: 1) a plain at 200–400 m, 2) a first plateau sloping northwest at 400–700 m, 3) a second plateau at 700–900 m, and 4) mountains between 900 and 1,400 m (Figure 1).

Composition of the agricultural land. Land usage ratios were derived from the 1988 General Agriculture Census, and the National Forest Census, both from the Ministry of Agriculture. The variables used in this study were the ratios permanent grassland/total land, plowed fields/total land, and the forest/total land.

Climatic characteristics. Echinococcus multilocularis is known to be a cold climate parasite whose eggs are very sensitive to moderate temperature and dessication. It has been emphasized that its distribution range is limited by temperature and rainfall at a regional scale. Moreover, in the Doubs Département transmission of M. arvalis usually occurs during autumn and winter but not in spring and summer (Giraudoux P, 1991. Hosts of Echinococcus multilocularis and Space Use: Epidemiological Consequences. PhD dissertation. Laboratory of Ecology, University of Burgundy, France). Thus, climatic characteristics play an important role in transmission at both the regional and local scales and had to be considered as potential confounding factors. Data on monthly average temperatures and rainfall were obtained for a 24-year period from the Regional Meteorological Department. They have been averaged on this whole time period, but split to distinguish cold (October to April) from warm (May to September) periods. Thus, 4 indices were constructed: cumulative winter rainfall, cumulative summer rainfall, winter temperature, and summer temperature.

Estimation of vole densities. The fossorial form of the water vole (A. terrestris scherman) causes extensive damage associated with serious economic losses in Franche-Comté, mainly in grassland areas. Three outbreaks of these intermediate host populations have been reported in the Doubs Département from 1979 to 1995 with a similar geographic...
pattern. However, the 1989–1995 wave considered in this study is the only one to have been monitored with standardized sampling procedures. There is no easy method to estimate water vole densities and distribution at regional scale, apart from an index method. Data were collected every year by the technicians of the Regional Crop Protection Service, Ministry of Agriculture who visited 80% (in 1993) to 97% (all other years) of the communes in the Département to record vole colonies, which were split in three categories: <100 individuals/hectare, between 100 and 200 individuals/hectare, and >200 individuals/hectare (see Giraudoux and others for more details).

To handle these exposure estimations, a composite index has been created as follows. For each commune, the number of years when the vole density was beyond 200 individuals/hectare was first cumulated during this 7-year period. A weighted mean was then calculated for each canton, with the surface of each commune composing the canton, as weight. Finally, this weighed mean was divided by 7 to obtain a score ranging from 0 to 1, yielding a vole density index.

**Case identification.** The AE identification process has been carried out for many years by the World Health Organization Collaborating Center for Prevention and Treatment of Human Echinococcoses at the University Hospital of Besançon, the only regional referring hospital for this pathology. Data were retrospectively compiled in 1988, and subsequently updated in a prospective way. Data sources consisted of hospital records (hepatology, abdominal surgery, ultrasonography departments), laboratory records, and minimum inpatient data sets. Ethical clearance for this study was granted by the Protection of Human Subjects in Biomedical Research Committee of the Franche-Comté region. All patients provided informed consent regarding the use of their hospital records for research purposes at the time of their diagnosis.

To rely on irreproachable record keeping, cases prior to 1980 were discarded. The study period covered the years up to 1992, which was the last year of quality-checked records at the time of analysis. All records were carefully reviewed for the purpose of this study. In particular, we have not included asymptomatic cases detected by a seroepidemiologic screening survey carried out between 1987 and 1993 in the Doubs Département. As a result, 13 asymptomatic AE patients (including 5 abortive cases) were identified by immunodiagnostic ELISAs combined with modern imaging methods. However, since this screening was restricted to volunteers from the sole Agriculture health insurance group (covering about 10% of the population), a differential bias was possible. Thus, only diagnostic incidence data were considered in this study.

**Statistical analysis.** Units considered in the statistical analyses consist of the 29 electoral wards or cantons (2,900–123,000 inhabitants, 50–325 km²) that composed the Doubs Département. However, due to changes in the definitions of boundaries between the 1982 and 1990 censuses, which resulted in new cantons partially overlapping some old ones, we were forced to aggregate some of them to obtain unequivocal and stable spatial units across years. Thus, 26 statistical units rather than 29 original cantons are considered in this study.

Expected numbers of cases for each canton were computed by applying an internal standard (i.e., incidence rates from the entire Doubs Département for the same years) to the person-years of each area stratified by gender and 5-year age classes. Population data by canton, gender, and 5-year age groups were obtained from the French Office of Population Censuses for the three censuses of 1975, 1982, and 1990. Linear interpolation between these populations was used to estimate the person-years for the period 1982–1990, while linear extrapolation was applied for the years 1991 and 1992.

Models were fitted to the grouped data with Poisson regression analysis. Logarithms of observed and expected cases were linked with a set of covariate values in a linear model that accounted for Poisson errors. Land usage ratios, vole density index, and climatic characteristics were fitted as continuous variables in the model. Geomorphologic features were fitted as two dummy variables, namely, first plateau and second plateau (the latter also including mountains represented by only one canton), with plain serving as a reference. The Poisson model equation is log(O_i) = log(E_i) + m + x_i b, in which i = 1−26, O_i = observed number of cases, E_i = expected number of cases, m = grand mean, x_i = row vector of regression variables, and b = column vector of regression coefficients.

To model the covariate effect in the presence of both unstructured and geographically autocorrelated extra-Poisson variations, an approach originally developed by Clayton and others was adopted. This extra-Poisson regression model may be written as log(O_i) = log(E_i) + m + x_i b + e_i(1) + e_i(2).

The first component of variation, e_i(1), is the spatially unstructured extra-Poisson variation, also called heterogeneity. These random effects are independent and normally distributed. The second component of variation smoothly changes across areas and is called clustering. These random effects are normally distributed, and their respective means are given by the means of geographically adjacent neighboring e_i(2).

This method addresses the issue of spatial autocorrelation. Modeling the heterogeneity variation accounts for unmeasured explanatory variables, which vary between areas in an unstructured way, whereas modeling the clustering variation accounts for those unmeasured risk factors that smoothly vary with location. One may note that when random effects are not included in the model, it is simply a Poisson regression. In both models, estimates of relative risks (RRs) for independent variables are provided by exp(b).

To interpret how the risk of human AE changes with the continuous scaled covariates, RRs are reported for the upper quartiles of exposure (with the lowest quartiles as control groups), with their corresponding 95% confidence intervals (CIs). Univariate analyses were first carried out. Variables were included in the multivariate analysis if they had a P value ≤ 0.20 in the univariate approach. All analyses were performed with BEAM software.

**RESULTS**

Forty-nine cases of human AE accrued between 1980 and 1992. Figure 2 shows the standardized incidence ratio (SIR) versus vole density index in 26 cantons, with different symbols according to their altitude category. No cases were ob-
served in 4 cantons, whereas the maximum number of cases reached 9 in 1 area (versus 1.33 expected) yielding an SIR = 6.75. However, the highest SIR was observed in another canton (O = 6, E = 0.67, SIR = 8.9), corresponding to a crude incidence rate of 91.7 per 100,000.

Two main features emerge from Figure 2. First, a global positive trend is noticeable, despite one outlier (SIR = 0, vole density index = 0.235). However, the latter is associated with a low expected count (0.50) and therefore with a highly unstable SIR. Second, the geomorphologic variable seems to be discriminating, with the plain exhibiting a low combined SIR (0.40, 95% CI = 0.22–0.66), the second plateau an intermediate SIR (2.29, 95% CI = 1.40–3.54), and the first plateau the highest SIR (4.79, 95% CI = 2.62–8.04).

Univariate analyses are summarized in Table 1. Seven variables appear as significant risk factors: permanent grassland/total land (RR = 3.73, 95% CI = 2.49–5.60), vole density index (RR = 4.15, 95% CI = 2.83–6.09), cumulative winter rainfall (RR = 3.69, 95% CI = 1.12–12.08), cumulative summer rainfall (RR = 5.50, 95% CI = 1.39–21.87), winter temperature (RR = 0.38, 95% CI = 0.15–0.98), summer temperature (RR = 0.30, 95% CI = 0.11–0.83), and geomorphologic features (first plateau: RR = 11.82, 95% CI = 5.31–26.30; second plateau and mountains: RR = 5.75, 95% CI = 2.73–12.10).

Results of the multivariate approach are shown in Table 2. Two risk factors remain significantly associated with human AE. The vole density index yields an increased risk compared with the univariate approach (RR = 10.34, 95% CI = 2.78–38.39). Although still significant, the risk associated with the first plateau decreased (RR = 7.10, 95% CI = 1.30–38.63). A significant heterogeneity effect is noticeable (P = 0.04).

**DISCUSSION**

The results of this study provide support for the influence of rodent densities on human AE since a significant link was
Table 2
Relative risk (RR) estimates of human alveolar echinococcosis (49 cases, multivariate analysis, Doubs Département, France, 1980–1992)

<table>
<thead>
<tr>
<th>Exposure variable</th>
<th>RR*</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permanent grassland/total land</td>
<td>1.36</td>
<td>0.55–3.37</td>
<td>0.51</td>
</tr>
<tr>
<td>Vole density index</td>
<td>10.34</td>
<td>2.78–38.39</td>
<td>&lt;10⁻³</td>
</tr>
<tr>
<td>Cumulative winter rainfall</td>
<td>1.30</td>
<td>0.15–11.65</td>
<td>0.81</td>
</tr>
<tr>
<td>Cumulative summer rainfall</td>
<td>0.03</td>
<td>0.00–3.46</td>
<td>0.15</td>
</tr>
<tr>
<td>Winter temperature</td>
<td>0.07</td>
<td>0.00–17.71</td>
<td>0.35</td>
</tr>
<tr>
<td>Summer temperature</td>
<td>2.00</td>
<td>0.00–27.78</td>
<td>0.85</td>
</tr>
<tr>
<td>Geomorphologic feature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>First plateau</td>
<td>7.10</td>
<td>1.30–38.63</td>
<td>0.03</td>
</tr>
<tr>
<td>Second plateau and mountains</td>
<td>0.73</td>
<td>0.09–5.59</td>
<td>0.76</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>–</td>
<td>–</td>
<td>0.04</td>
</tr>
<tr>
<td>Clustering</td>
<td>–</td>
<td>–</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* Relative risk estimates are reported for the upper exposure quartiles, using the lowest quartiles as control groups.

The water vole density index used in this study is composite, which impedes the identification of any dynamic (propagation speed) or temporal (interannual fluctuations) patterns. Moreover, A. terrestris may not be the sole species to play a role, and could also partially represent a surrogate of M. arvalis densities (of which the outbreak ROMPA threshold is lower than that of A. terrestris). Unfortunately, it was not possible to monitor other species at such a large scale during the study period. Thus, the index should be considered as an overall, though carefully evaluated, quantification of small mammal densities.

The extra-Poisson variation model seems to be an attractive tool to deal with data collected at an area level. It represents a way of accounting for unmeasured variables that differ between areas in an unstructured manner (random heterogeneity), and for unmeasured risk factors that vary locally (clustering random effect). Thus, deviations from Poisson distribution and spatial autocorrelation were taken into account.

The issue of how landscape can drive population dynamics of small mammals has been largely discussed from a theoretical point of view, and supported by some empirical evidence for M. arvalis and A. terrestris, two grassland species. Relationships between land characteristics (grassland, beech series) and distribution of E. multilocularis in western Europe have been pointed out for foxes. High human AE prevalence rates have also been reported in the areas that exhibited a high ratio of permanent grassland. Further, providing more evidence in favor of rodent populations of A. terrestris as a risk factor for human AE, while distinguishing its effects from those of climate, altitude, land usage, vegetation, and spatial autocorrelation. In this respect, the first plateau has captured the effects of all climatic and land composition variables introduced into the multivariate analysis. Its intermediate temperature, rainfall, and permanent grassland surface (in comparison with plains on the one hand, and second plateau and mountains on the other), seem to contribute to an ecosystem favorable to either the survival of E. multilocularis eggs or their transmission, or both. A significant random heterogeneity is also noticeable, indicating that some unknown or unconsidered factors can play a role. This pattern seems characteristic of E. multilocularis distribution at several scales. Indeed, highly variable prevalence rates in humans between villages in an area of high endemicity in central

This study goes a step further, providing more evidence in favor of rodent populations of A. terrestris as a risk factor for human AE, while distinguishing its effects from those of climate, altitude, land usage, vegetation, and spatial autocorrelation. In this respect, the first plateau has captured the effects of all climatic and land composition variables introduced into the multivariate analysis. Its intermediate temperature, rainfall, and permanent grassland surface (in comparison with plains on the one hand, and second plateau and mountains on the other), seem to contribute to an ecosystem favorable to either the survival of E. multilocularis eggs or their transmission, or both. A significant random heterogeneity is also noticeable, indicating that some unknown or unconsidered factors can play a role. This pattern seems characteristic of E. multilocularis distribution at several scales. Indeed, highly variable prevalence rates in humans between villages in an area of high endemicity in central

Insurance contributors, who were more numerous in rural areas than in urban ones, might have yielded higher observed counts in the countryside and then affected estimation of regression coefficients. Nevertheless, to check this assumption we have performed a sensitivity analysis, including the 13 asymptomatic cases in a multivariate model composed of the same covariates. The results turned out to be very similar, underscoring the same two significant factors: vole density index (RR = 7.17, 95% CI = 2.33–22.05), and first plateau (RR = 10.04, 95% CI = 2.24–44.97). This stems from the occurrence of asymptomatic cases in those cantons that had already been identified as areas of high incidence. Thus, the results are not explained by urban/rural contrast, but by orthogonal partition based on rodent densities or surrogates.

Geographic studies are a natural design for the study of an environmental factor since they are less prone than individual epidemiologic studies to the effects of random error in the measurement exposure. Moreover, in our case the factors considered could only be accurately assessed at the ecologic level, and it might be difficult, if not impossible, to address this question of environmental health using other approaches. However, as in any spatial regression analysis, this one could be subject to ecologic fallacy, with the relationship between exposure and disease in groups not necessarily being the same as in individual people. Ecologic fallacy is more likely to occur when populations or geographic units are broad, when migrations are observable, when exposure is recorded at the same time as the outcome, or when the accuracy of exposure measurement is questionable. In our opinion, cantons represented a good trade-off, large enough to give rise to a sufficient number of events (insuring a reasonable statistical power), and small enough to limit ecologic fallacy. Nevertheless, we acknowledge that among the different exposures under study, rodent densities were recorded during the same time period as cases of human AE. We have indeed favored the accuracy of measurement at the expense of the strict respect of a time lag, aware that the last 3 outbreak waves of A. terrestris that have been observed in the Doubs Département showed very similar patterns. Finally, the residential stability of local populations, as well as the use of statistical models accounting for autocorrelation, further contributed to attenuate ecologic fallacy.

Regarding the outcome, we have focused on diagnostic incidence, excluding seroepidemiologic prevalence data. Our goal was two-fold. The first was to allow international comparisons between population-based estimates. In this respect, cumulating diagnostic cases occurring in the whole population with asymptomatic cases screened in an agricultural fraction of the latter would have been misleading (even if the incidence of AE among non-agricultural workers is probably low). The second was to avoid any differential bias. Considering screening data from the sole agricultural health...
China, which could not be explained by landscape contrasts, have already been described.\textsuperscript{2,23}

High density of grassland species could act by fostering transmission through the prey/predator relationships. Indeed, in periods of high density, foxes become specialized and feed almost exclusively on grassland species (functional response).\textsuperscript{8} It can be hypothesized that this could lead to higher prevalence rates and higher worm burden in foxes and therefore to more infective material dispersed in the environment, including the close vicinity of human settlements.\textsuperscript{8,3} As a subterranean species, \textit{A. terrestris} build tumuli that can cover almost 100\% of certain meadows in late winter. This fossorial activity may consequently favor the burying of \textit{E. multilocularis} eggs into the soil, which protects them from heat and desiccation. Moreover, local people usually collect young dandelions (\textit{Taraxacum officinale}) for salad on those tumuli and therefore can be contaminated (all the more likely since foxes mark their territory by preferentially defecating on bare ground)\textsuperscript{24}).

The major cause of an increase in the amplitude of the pluriannual density fluctuations of water vole in the Doubs Département is probably due to the agricultural policy of the 1960s, which led farmers to specialize in milk production and convert plowed fields into permanent grassland.\textsuperscript{6,7} Water voles have caused heavy losses in grass production and local farmers have developed control methods since the 1970s. These consist of anticoagulant rodenticides used at the plot scale, which were unsafe for the auxiliary wild fauna (especially foxes and buzzards). Thus, local authorities and farmer organizations are currently considering alternate methods applied at the regional scale and based on changes in grass production (such as shorter plowing rotation and landscape management limiting open areas).\textsuperscript{6} In light of this study, one hopes for a long-term decrease in AE incidence as a positive side effect of rodent pest control on a regional scale.

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