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GEOGRAPHIC INFORMATION SYSTEM-AIDED ANALYSIS OF FACTORS ASSOCIATED WITH THE SPATIAL DISTRIBUTION OF *ECHINOCOCCUS MULTILOCULARIS* INFECTIONS OF FOXES

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Abstract. To investigate the influence of environmental factors on the spatial epidemiology of infections with *Echinococcus multilocularis*, foxes were sampled in a focal endemic region in the Northwest of Brandenburg, Germany, and examined for infection by the parasite. The locations where foxes were obtained were recorded in a geographic information system database. Positions of infected and uninfected foxes were analyzed on the background of geographic vector data of water, settlements, streets, forests, crop, and pasture. Fox positions were allocated to different land-use classes by use of a Landsat Thematic Mapper (TM) satellite image. Infected foxes were more frequently shot near water, in areas of high soil humidity, and on pastures, suggesting that dryness may limit the tenacity of *E. multilocularis* oncospheres. Thus open landscapes with humid soil seem to be favorable for the life cycle of the parasite. In contrast, infected foxes were significantly underrepresented in forest areas.

INTRODUCTION

Alveolar echinococcosis, caused by the larval stage of *Echinococcus multilocularis*, is considered as the most dangerous autochthonous parasitic zoonosis in central Europe. The obligate 2-host parasitic cycle of *E. multilocularis* is predominantly sylvatic. In Europe, the red fox (*Vulpes vulpes*) is the main definitive host. Several rodent species are intermediate hosts in the parasite life cycle. Humans become infected by ingestion of oncospheres and rarely are intermediate hosts, but precise risk factors for human infection are largely unknown.[1] As a consequence, a risk for human infection must be expected wherever the parasite is detected in foxes, even if there are no reports about cases of alveolar echinococcosis in that particular region. To assess potential risk areas, it is important to know the spatial distribution patterns of the parasite and factors influencing these patterns.

A previous study has shown that *E. multilocularis* is heterogenously distributed within an endemic area in northwestern Brandenburg, Germany. There are scattered foci in the endemic area with an estimated prevalence of ∼ 25% and a peripheral area with an estimated prevalence of ∼ 5%, with prevalence estimates performed for municipalities as the geographic unit.[2] Because it is known that elevated temperature and desiccation can effectively reduce oncosphere infectivity, microclimate and habitat can be suspected as factors that potentially influence spatial heterogeneity of the parasite.[3] To investigate these environmental conditions in an endemic area in the northwest of Brandenburg, Germany, foxes were randomly sampled and examined for infection with *E. multilocularis.* The geographic positions of the locations where foxes were shot were entered into a geographic information system (GIS) database and analyzed for potential associations with the following: topographic features (water, streets, settlements); soil humidity (on the basis of a Landsat TM satellite image); and land-use classes.

MATERIALS AND METHODS

Sampling and parasite data. Foxes (n = 3,797) sampled between 1995 and 1997 were collected and necropsied as described from the 2 northwestern counties Prignitz and Ostprignitz-Ruppin, which are situated 11.5–13° N and 52.5–53.5° E and cover an area of 4,450 km.[2,2] The detection of adults of *E. multilocularis* was performed according to the intestinal scraping technique, a World Health Organization standard method.[4] Legal hunters who killed the foxes to support nature conservation were asked to mark the location where each fox was shot on a topographic map (scale 1: 50,000) and to name the hunting district for cross-checking.

Geographic information system database. The geographic positions where the foxes had been shot were digitized on-screen as point coverages with the GIS tool ArcView 3.1 (ESRI; Redlands, CA). A relational database was created that included for each sampled animal the result of the examination for *E. multilocularis* and the geographic position where the fox had been shot. Raster- and vector-based analyses were carried out in ArcView, ARC/INFO 7.2.1 (ESRI) and Imagine 8.3 (ERDAS; Atlanta, GA). Vectorized data of lakes and rivers, villages, streets, and forests were used to describe the topography of the landscape on the basis of the geological survey of the Federal State of Brandenburg (TK 50; reference map scale: 1:50,000; GB-D 27/94; May 1995). A digital terrain model of the study area was generated from the vectorized contour lines and elevation points in ARC/INFO by use of the TopoGrid algorithm. To identify land-use classes with higher resolution, vector data of the CORINE (Co-ORdination of Information on the Environment) Land Cover project were used.[5] Computerized thematic maps on 44 different land cover categories were generated on the basis of satellite images of Landsat TM and KFA 1000, topographic maps (TK 50 and TK 100) and panchromatic aerial photographs. The minimal digitized land cover unit comprised an area of 25 hectares (ha). For the purpose of this study, the original 44 CORINE Land cover (CLC) categories were combined into 4 classes: urban/water, crop, pasture, and forest.

To identify soil moisture variation, a georeferenced Landsat 5 TM satellite image (185 × 175 km) with 25-m resolution, recorded on October 6, 1994, was obtained from the

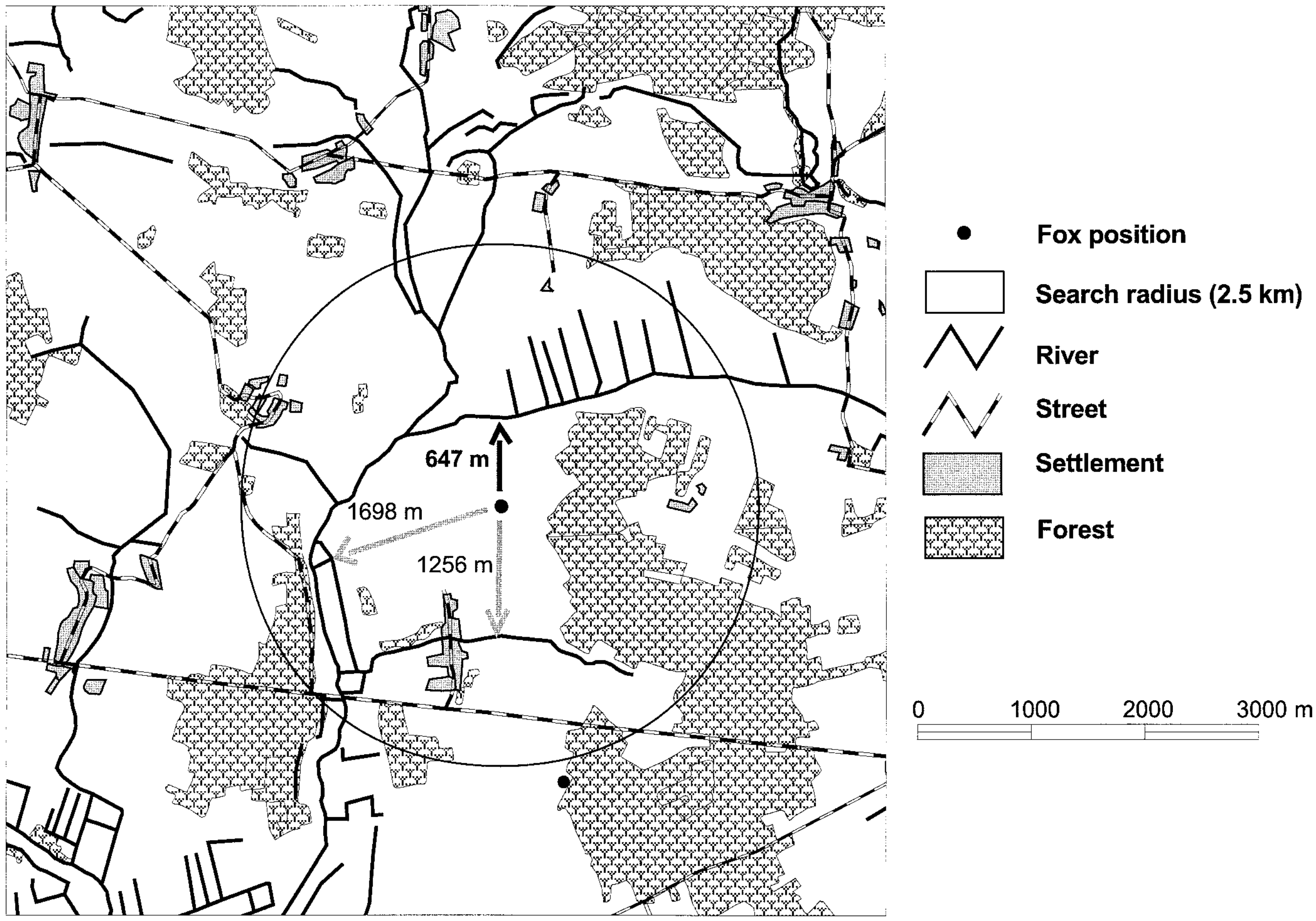

FIGURE 1. Neighborhood analysis. Identification of topographic features associated with the geographic positions of foxes infected with *Echinococcus multilocularis.* We used geographic information system analysis to calculate the distance from each digitized fox position to the nearest feature in a topographic category. Therefore, the algorithm measures the distances, in this example, to every river within the search radius of 2.5 km. The shortest distance is then recorded in the database for each fox.

German Remote Sensing Data Center. The Kauth-Thomas tasseled cap transformation of a satellite scene was used to separate the spectral data variation into brightness, greenness, haze, and wet data structures.[6,7] To minimize false classification as tree canopy in the wetness image,[6,8] the area of forests, lakes, rivers, fish ponds, and the like was either automatically erased by overlay analysis with the vector data (TK 50) or corrected by hand by the image processing software Imagine or the Spatial Analyst 1.1 module of ArcView. The correctness of the TM tasseled cap wetness dimension (very dry to wetland) was verified by expert knowledge, field examination, and slope data derived from the digital terrain model to identify sinks, depressions, and flat areas. To minimize errors in overlay or spatial analysis, all vectorized data sets were re-projected onto the Landsat TM image (Universal Transverse Mercator, zone 32).

Spatial analysis. The digitized fox positions and the vector data of the geological survey of Brandenburg were used to calculate the relative position of each sampled fox to the different topographic categories—that is, the distance to the nearest line feature in the coverages of rivers and streets or to the next border line in the polygon coverages of lakes, settlements, and forests (neighborhood analysis, Figure 1). The search radius was restricted to 2.5 km, which was assumed to represent the average home range of foxes in the study region on the basis of telemetric studies in a nearby area with a very similar landscape structure. (Stiebling U, unpublished data).[2]

To account for the area of the fox home ranges, overlapping buffer zones with a radius of 2.5 km were created for each sampled fox in ArcView. The habitat characteristics of the buffer zones were analyzed for wetness and land use. A wetness index was calculated for each fox by averaging the wetness values over all cells of the TM tasseled cap grid covered by the respective buffer zone. To determine the land-use composition of the buffer zones, an overlay analysis was performed by intersecting the buffer zones with the CLC map of land-use categories (Figure 2). In addition, the relative land-use composition of the buffer zones was calculated for 25,000 randomly generated geographic positions, excluding the category urban/water.

Computing and statistics. A program written in CLIPPER (Computer Associates International, New York, NY) was used for the documentation of the fox data in a dBASE file (5.0 for Windows, Borland International, Scotts Valley, CA). Statistical analyses were performed with S-Plus 2000 and S-Plus for ArcView 1.1 (Mathsoft, Seattle, WA). The distributions of distances of sampled animals from the to-

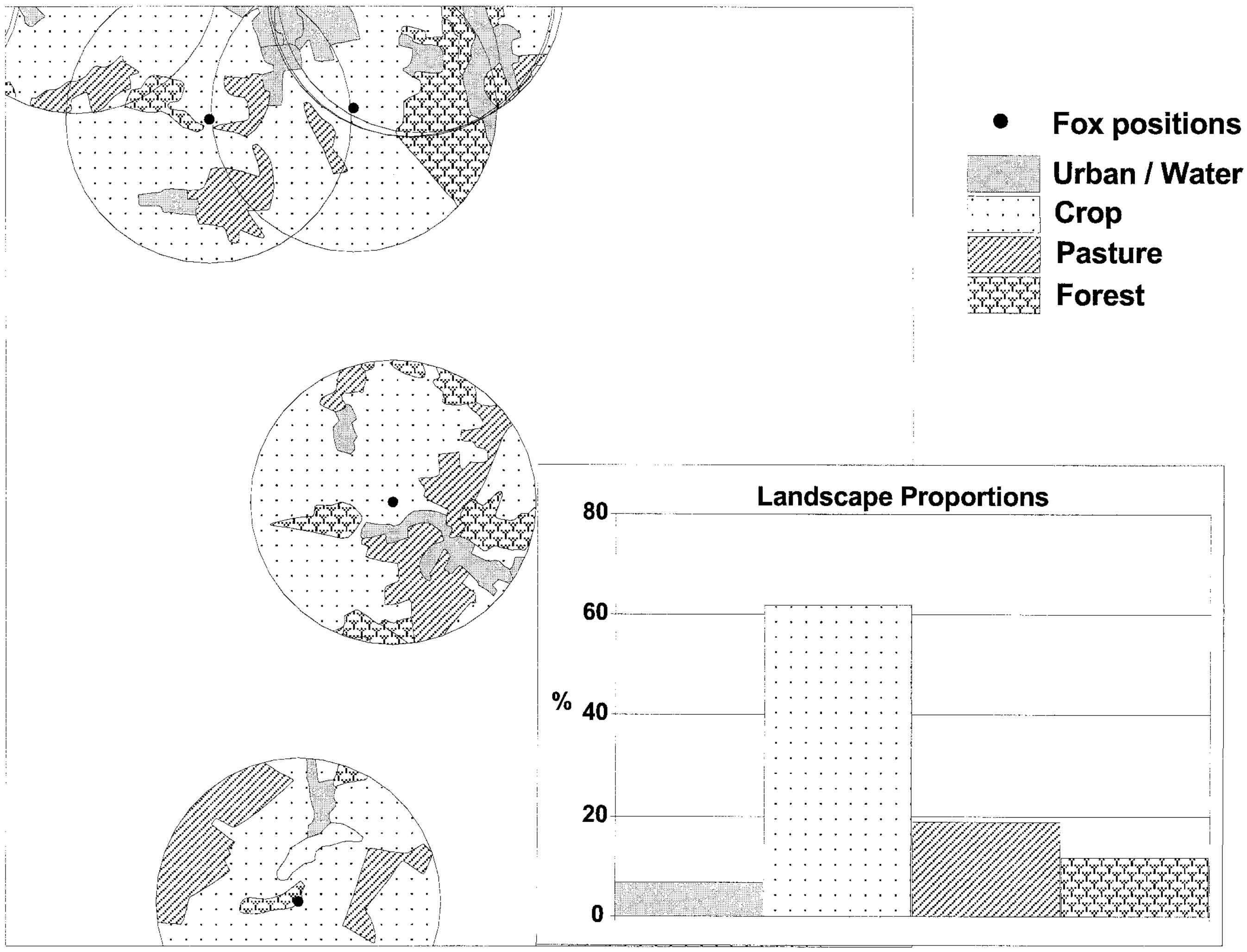

FIGURE 2. Overlay analysis. Identification of land-use classes associated with the geographic positions of foxes infected with *Echinococcus multilocularis.* Buffer zones with a radius of 2.5 km around each digitized fox position were intersected with the CORINE Land cover coverage to determine the classes for the average home range of a fox in the study area. Also shown are the calculated landscape proportions for the buffer zone of the digitized fox position in the center of the main view **(inset).**

pographic categories and the distribution of wetness indexes were analyzed by the Mann-Whitney *U*-test. The relative composition of the buffer zones with regard to land-use categories were compared by the chi-square test.

RESULTS

During the investigation period, complete data sets (n = 3,521) for sampled foxes were obtained. Eighty-three foxes were found to be infected with *E. multilocularis,* giving a prevalence of 2.4% in the random sample (Table 1).

TABLE 1
Distribution of *Echinococcus multilocularis* among foxes in the random sample

	Infection with *E. multilocularis*		
Sex	Yes	No	Total
Female	37	1,561	1,598
Male	46	1,877	1,923
Total	83	3,438	3,521

Mean and median distances of the positions, where the sampled foxes had been shot, to the topographic categories rivers, lakes, forests, villages, and streets were compared for *E. multilocularis*-infected and uninfected foxes (Table 2). This analysis showed that the positions where *E. multilocularis*-infected foxes had been shot were significantly closer to items of the category of rivers compared with the positions of foxes found uninfected (Mann-Whitney *U*-test, P = 0.0048). For the remaining topographic categories, the respective distances of the positions of infected and uninfected foxes were not statistically different.

Comparison of the wetness indexes calculated for each buffer zone showed significantly higher values (i.e., higher mean humidity in the buffer area) for infected than for uninfected foxes (Mann-Whitney *U*-test, P = 0.013; Figure 3).

When the proportion of each land-use category was quantified for each buffer zone and the results compared for *E. multilocularis*-infected and uninfected foxes, the buffer zones of infected foxes contained an increased proportion of pasture (26.8%) and a decreased proportion of forest (14.3%), whereas foxes found to be uninfected with *E. mul-*

TABLE 2
Distances of the positions of shot foxes to the nearest polygon or line of a topographic category

Topographic category	Number of analyzed foxes*	Infected, *n* (%)	Mean distance (m)		Median distance (m)		*P* value†
			Infected	Uninfected	Infected	Uninfected	
River	3,360	83 (2.5)	239	341	149	237	0.0048
Lake	2,086	54 (2.6)	630	671	645	685	NS
Forest	2,819	70 (2.5)	402	332	279	194	NS
Village	3,293	78 (2.4)	545	579	512	549	NS
Street	3,384	80 (2.4)	586	523	532	465	NS

* Differences in the numbers of analyzed data sets results from the limitation of the search radius to 2.5 km.
† NS = not significant ($P > 0.10$).

tilocularis had buffer zones with a lower proportion of pasture (17.8%) and a higher proportion of forest (25.7%, chi-square test, 5.1, df = 2, $P = 0.078$; Table 3).

When relative frequencies of land-use classes were determined for all positions where infected and uninfected foxes had been shot, a strong deviation from the landscape-determined proportions was found (crop, 57.2% [all fox positions] versus 49.8% [CLC data]; pasture, 18.2% versus 15.5%; forest, 23.8% versus 29.8%), indicating a preference of the hunters for open areas. However, when relative proportions of land-use classes in all buffer zones were compared with those of 25,000 random positions generated in the GIS, the respective distributions were not statistically different (chi-square test, 0.490, df = 2, $P = 0.783$). Therefore, the buffer zones provide a random sample from the actual landscape concerning the distribution of the land-use classes. Hence, the preferences of hunters influencing the original shooting points were adjusted at the buffer zone level, which permitted the following analysis. In contrast, the distribution of the proportions for *E. multilocularis*-infected foxes was significantly different compared with the randomly generated positions (chi-square test, 7.431, df = 2, $P = 0.024$). The stratified sample of those foxes that were not found infected with *E. multilocularis* did not deviate from random (chi-square test, 0.263, df = 2, $P = 0.877$).

DISCUSSION

GIS analysis was used to analyze the association of *E. multilocularis*-infected foxes with topographic features—wetness and land-use—of an area endemic for the parasite. Such analysis allows graphic and statistical analysis of the spatial distribution of diseases and of potential associations with topographic, environmental, or ecological parameters.[9] The GIS process uses spatial data in the form of cartographic information (maps) describing the location of features with textual attribute data on the characteristics of such features in a relational data base.

It is known that elevated temperature and aridity have a substantial influence on the tenacity *E. multilocularis* oncospheres in the environment.[3] Studies conducted in France suggest that vegetation types, and geological and climatic factors influence the spatial distribution of *E. multilocularis* transmission.[10,11] Because *Microtus arvalis* is an important intermediate host for *E. multilocularis* in France, it is of interest that a correlation of microtine rodent indexes and land-use variables was observed.[12] It has also been reported from France that a high density of voles (*Arvicola terrestris*) and certain geomorphological and climatic conditions could be potential risk factors for the acquisition of human alveolar echinococcosis.[3] The authors of these studies argued that in periods of high vole densities, foxes may feed almost exclusively on grassland rodents (including *A. terrestris*), and thus hypothesized that this might lead to higher prevalences and infection intensities of *E. multilocularis* in local fox populations, finally leading to increasing contamination of the environment with infective parasite oncospheres. It is rather questionable, however, whether quantitative fluctuations in vole abundance can lead to the extent of spatial heterogeneity of *E. multilocularis* infections in foxes that has been observed in our study area.[2] Among the geomorphological and climatic factors, intermediate temperature, rainfall, permanent grassland surface, and some other factors were suspected to contribute to an ecosystem favorable for survival and transmission of *E. multilocularis.*[13] Previous investigations conducted in the same area as the present study yielded indications that soil humidity, topographic features, and land-use can influence the prevalence of *E. multilocularis*-infected foxes.[3] Moreover, small differences in the suitability of a habitat for the survival of oncospheres or their chance to infect an intermediate host were shown to be essential for the persistence of the life cycle of the parasite.[14] Thus, the aim of the present study was to identify risk factors that may be related to the locally variable influence of temperature and humidity and aridity in the field by a GIS database and statistical analysis.

This study showed that *E. multilocularis*-infected foxes were more frequently shot in the proximity of rivers, brooks, and other types of flowing waters. By contrast, none of the remaining topographic categories tested yielded a statistically significant difference between infected and uninfected foxes. Moreover, the positions of *E. multilocularis*-infected foxes were associated with buffer zones with an elevated wetness index. These results are consistent with the reported sensitivity of the oncospheres of the parasite to dryness. Thus, humid soil conditions may be favorable for the persistence of the parasite's life cycle in a habitat. On the other hand, this habitat is preferred by muskrats (*Ondatra zibethicus*), which also represent a susceptible intermediate host species.[15,16] High prevalences at the local level may be associated with the longer life span of the muskrat when compared with microtine life expectancy. However, the importance of muskrats as a food source for foxes (as the basis for an infection risk) is doubtful. Rather muskrats appear to be bioindicators for a local infection risk for intermediate hosts. Thus, the association of the infection risk with humid microclimatic conditions for the oncospheres seems more plausible.

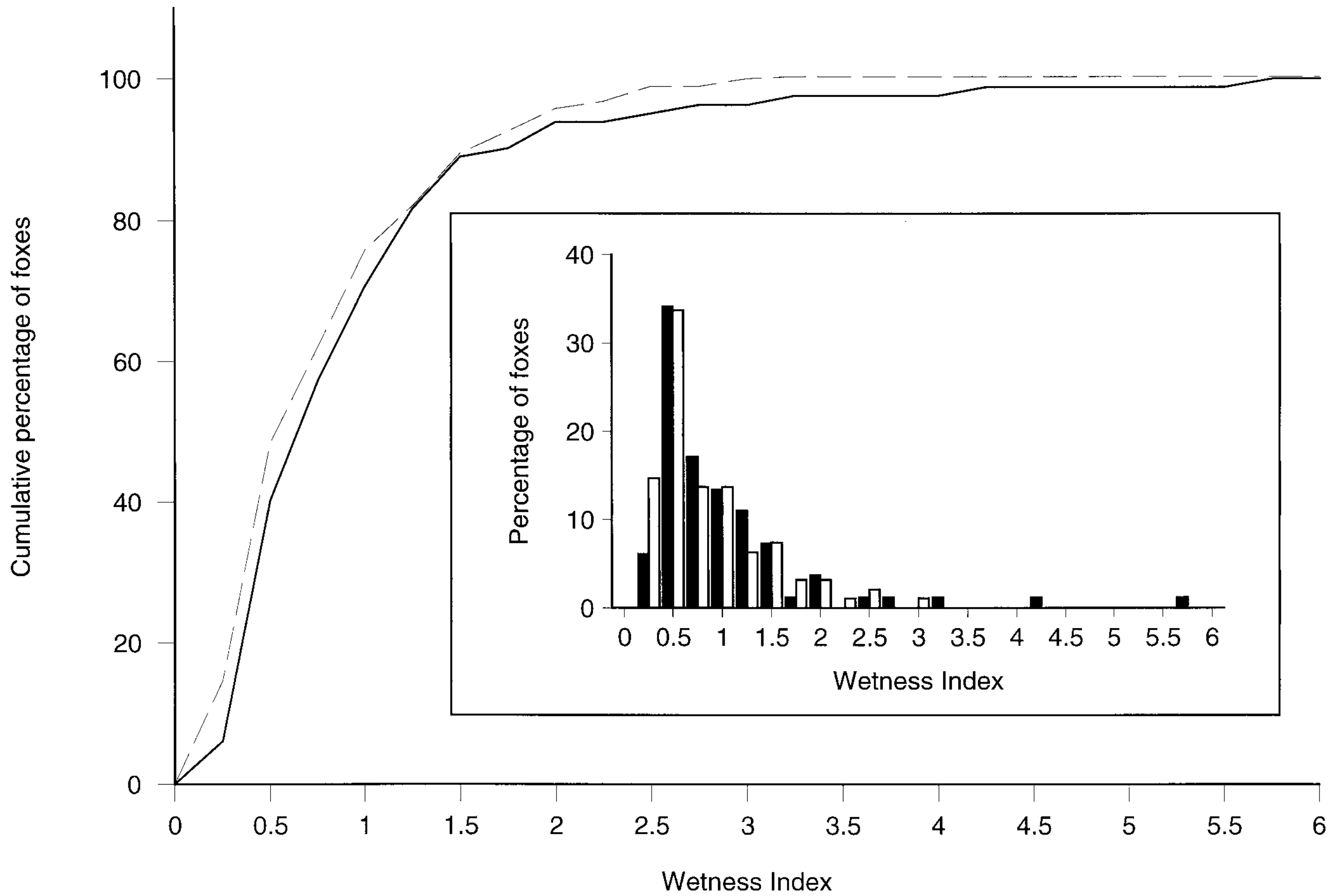

FIGURE 3. Distribution of the wetness indexes for infected and uninfected foxes. The wetness index was determined for the buffer zones (2.5-km radius) around the positions where each fox had been shot. The histogram shows the absolute percentage of foxes infected with *Echinococcus multilocularis* **(dark bars)** and uninfected foxes **(light bars)** for the respective wetness index class. In the line diagram, the cumulative percentage of *E. multilocularis*-infected and uninfected foxes is plotted against the respective wetness index classes to illustrate the differences between the distributions.

An overlay analysis that tested potential associations between the positions of *E. multilocularis*-infected foxes with land-use classes indicated that the buffer zones of infected foxes contained a significantly higher proportion of the land-use class pasture, whereas the proportion of forest was significantly lower. This analysis suggests that *E. multilocularis*-infected foxes would be expected more frequently in a pasture-like landscape in the study region, if our assumptions regarding the expected home ranges of foxes are true. Moreover, the calculations were also performed for other radii of the buffer zone (i.e., 1, 5, 7.5, and 10 km), but no major influence of the size of the home ranges on the result could be detected (Staubach C, unpublished data).

TABLE 3
Relative proportions of the land-use classes in a buffer zone of 2.5-km radius

Factor	Crop (%)	Pasture (%)	Forest (%)
Infected foxes	53.8	26.8	14.3
Uninfected foxes	51.9	17.8	25.7
Random sample	52.3	15.4	27.7
CORINE Land Cover data	49.8	15.5	29.8

The influences of the differences in hunter activity in the different land-use classes and the specific circumstances impacting the point positions where foxes were shot from could not be completely ruled out by the sampling design. But potential confounding was adjusted for by the buffer analysis that relates the stochastic shooting position of an individual fox to its likely spatial context with individual habitat features. Thus, the buffer zone data (not necessarily the actual locations of the shooting positions) obtained for all sampled foxes for 25,000 positions randomly generated in the GIS and the average CLC data for the study region did not differ with regard to the proportions of the land-use categories covered. The statistical analysis suggests that the hunters' preferences with regard to the land-use classes of the fox shooting positions did not change the environmental composition of the buffer. We thus conclude that the hunters' preferences did not confound the results of the overlay analysis presented here.

It is difficult to explain why the infection risk was lower in forest areas because humidity is expected to be higher in forests than in regions used for growing crops, and the lethal effect of ultraviolet rays on oncospheres may be lower. Perhaps a different population structure (e.g., species structure) of intermediate hosts, abundance of intermediate hosts, definitive hosts, or a combination of these factors are responsible for the lower infection risk for foxes shot in forests, possibly because of lower contact rates between the partners involved in the parasitic cycle (e.g., different feeding strategies of field and forest foxes). Also, forests are not particularly safe places for small rodents because there is a relative absence of cover at ground level. This would have an impact on species selection as well as on age profiles.

The finding that *E. multilocularis*-infected foxes were significantly underrepresented in forest areas may indicate that consumption of raw mushrooms and wild berries may be less important than commonly thought as a risk factor for human infection in the study area. Taken together with the data on the association with proximity to flowing waters and soil humidity, the association of the position of *E. multilocularis*-infected foxes with the land-use class pasture suggests that soils can be favorable for the life cycle of *E. multilocularis* where the oncospheres are protected from drying out as a result of soil humidity and the type of permanent vegetation.

Several approaches can be taken to confirm or deny the present findings. First, a predictive model could be developed to test whether the prevalence of infected foxes in regions yet to be analyzed is in fact related to land-use or topological features. This could be done retrospectively or prospectively. Similarly, where landscape modification is planned within already analyzed areas—for example, cleared forests or woodlands, changing drainage, hedge clearing, or regeneration—similar predictions could be made and then have the prevalence of *E. multilocularis* infection in foxes monitored. Last, the classification of vegetation in this study was coarse, with little discrimination among microenvironments. Four inclusive classes were derived from 44 classes of individual raster areas of 25 ha. The influence of ecological margins, buffer zones, and scale could be revisited for selected areas by use of the original Landsat TM data at a 30-m resolution to tease out more precise habitat contributors to parasite transmission. Furthermore, the study design could be used in field studies of other parasites with an environmental phase in the parasitic cycle, which is potentially influenced by heterogeneous habitat and microclimate conditions.[17]

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