

## Epidemiology of alveolar echinococcosis in southern Cantal, Auvergne region, France

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### Abstract

Alveolar echinococcosis (AE) is a helminth zoonosis which is encountered only in the northern hemisphere. In central France, the Auvergne region represents the most western and southern extension of this helminthiasis. In 1999, a human case of AE was diagnosed in the southern part of the Cantal department, where AE was supposed absent, and an epidemiological survey was subsequently carried out. The transmission of the zoonosis in the sylvatic and peridomestic definitive hosts was studied, as well as that in the rodent and human intermediate hosts. Eleven red foxes (*Vulpes vulpes*) were shot, and 50 fox faecal deposits were collected. Twelve farm dogs had their faeces taken by rectal touch, and four were checked after arecoline purgation. Optical detection of *Echinococcus multilocularis* worms was achieved on fox intestines after scraping, and also on dog stools after arecoline therapy. Coproantigen ELISA assay was performed for the 11 scraping products, for the 50 fox faeces, and for the 12 dog faecal samples. No adult AE agent was observed by microscopy, and the ELISA assay yielded positive results in one of 11 fox intestines, one of 50 fox faeces, and 2 of 12 dog faecal samples. Twenty-five small mammals were trapped, of which 19 were *Arvicola terrestris* water voles. One rodent liver exhibited a hepatic lesion consistent with AE. An epidemiological questionnaire was completed in 85 human volunteers, who were also serologically tested for AE. Only one (the case's husband) exhibited a Western-blotting pattern indicative of a low-grade AE infection. The results of this preliminary study suggested a slow AE extension to the south of Cantal department from the northern focus.

### Introduction

Alveolar echinococcosis (AE) is a helminth zoonosis due to the infection of small mammals by the metacystodes of *Echinococcus multilocularis*, a tiny tapeworm

parasite at the adult stage of the intestine of the definitive hosts, namely foxes, dogs and cats. Alveolar echinococcosis occurs exclusively in the northern hemisphere, within a belt descending from the Arctic zone to some areas around the 45th parallel. In the Old World, hot spots were found in Euro-Siberia and in China. In France, four endemic areas are currently recognized. According to data from EurEchinoReg ([www.eurechinoreg.org](http://www.eurechinoreg.org)), the

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European network for the surveillance of this disease, the most important focus, with a cumulative report of 130 cases from 1982 to 2001, encompasses the Doubs and the Jura departments in the Franche-Comté region, in the eastern part of France. Other endemic areas of lesser importance are centred on the Vosges massif, with 45 cases reported from 1982 to 2001, and on the Alpes mountains in the Rhône-Alpes region, with 37 cases reported from 1982 to 2001.

In the mountainous and volcanic centre of France, the Auvergne region represents for AE (15 cases reported from 1982 to 2001) the most western and southern endemic zone in Eurasia. Three cases were observed as early as 1977 (Rey *et al.*, 1977) and *Arolicola terrestris* was subsequently recognized as the most prominent intermediate host (Deblock & Pétavy, 1983). Two epidemiological surveys showed that the focus, in the Auvergne region (fig. 1), overlapped the Puy-de-Dôme and the Cantal departments (Pétavy & Deblock, 1983; Gilot *et al.*, 1988). Concerning the Cantal department, data from Gilot's study (1988) suggested AE was probably endemic only in the north of a NW/SE line, between the summits of the Puy Mary (elevation 1787 m) and the Plomb-du-Cantal (elevation 1855 m) peaks (fig. 1). This opinion was reinforced by the results of an epidemiological survey carried out in rural communities located N/NE of the Cantal volcano (fig. 1). This study was sponsored by the Farmer's Social Mutual Insurance (the French social security organization for rural people, best known by the French acronym MSA) and was based upon the detection of AE by both specific immunodiagnosis and liver ultrasonography in volunteers. According to an MSA's internal report (unpublished data), 506 volunteers participated in the survey. Of 393 immunodiagnoses performed using an ELISA test (brand name not available), none was found positive. Three hundred and forty-seven liver ultrasound examinations were done. Two cases of hepatic lesion consistent with AE were detected (0.56%), and 13 cases of intra-hepatic calcifications (3.75%) were reported. According to a state-of-the-art article dealing with this radiology topic (Paley & Ros, 1998), calcified granulomas and cystic echinococcosis are

the most frequent causes of this abnormality. Human hydatid disease is absent in the Auvergne region, and human fascioliasis exceptional above 600 m altitude. Alveolar echinococcosis could therefore account for the aetiology of some of these hepatic calcifications.

In 1999, an AE case was diagnosed at the University Hospital of Toulouse in a 78-year-old woman who dwelt in the hamlet of Billiez (45°6' N, 2°47' E), in the commune of Saint Martin-sous-Vigouroux (St Martin s/V.). The location of this case in a zone supposed AE-free until then, roughly 17 km south of the ecological barrier represented by the Cantal volcano area (fig. 1), prompted us to carry out the present study, the design of which was approved by the Board for Clinical Research of the Toulouse University Hospital. The purpose of this survey was to document *E. multilocularis* infection in the definitive and intermediate hosts, and to screen the residents of this zone, located beyond the supposed southern limit of the geographical distribution of the parasite in France, for AE infection.

## Materials and methods

### *Geographical and climatic characteristics of the studied area*

The St Martin s/ V. commune (1930 ha) is situated in the southern part of the Cantal volcano system, which covers 2500 km<sup>2</sup>. The geological limits are the volcano to the north, large old lava floods ('planèzes') to the east and west sides, and the reservoir of Sarrans Dam on the Truyère river to the south. The village (elevation 760 m) is on the western slope of a glacial valley oriented N/S, which has cut through the lava fields. The bottom of the valley is covered by meadows mowed in summer and by ploughed fields, the sides by beech woods (*Fagus sylvatica*), and the eastern and western flat ridges (the elevation of which ranges from 650 m to 1380 m) by permanent pastures. According to data from the meteorological station of Brommat (16 km to the SW, elevation 732 m), the average annual temperature is 9.6°C, and the average annual rainfall is 1276 mm.

### *The case*

In October 1999, a 78-year-old woman consulted her personal physician at the nearby city of Pierrefort (Cantal, France) due to the sudden onset of a pronounced jaundice. Primary laboratory investigations showed normal results for total and differential blood count, with specifically a lack of blood eosinophilia (228 eosinophils  $\mu\text{l}^{-1}$ ), both ALAT and ASAT results at five times the upper normal value and a total bilirubin dosage of 28 mg l<sup>-1</sup> (normal value < 10 mg l<sup>-1</sup>). The patient was referred to the Gastro-enterology Unit of the General Hospital in Aurillac, the prefecture city of the Cantal department, where ultrasound then computerized scan examinations showed a large mass in the right-hand part of the liver, along with central calcifications (fig. 2). This clinical, laboratory and radiology picture was consistent with an AE aetiology. The patient was then referred to the Department of Abdominal Surgery, Toulouse University Hospital, in order to confirm the diagnosis and to carry out palliative surgery. An immunodiagnosis of cystic

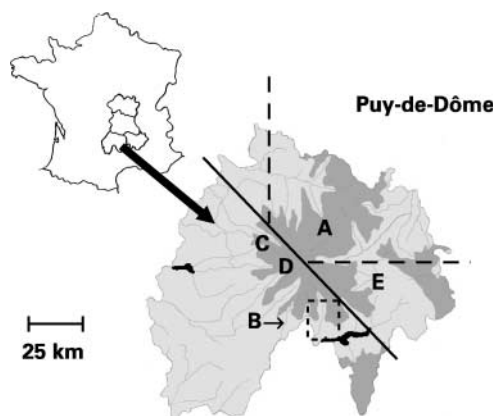


Fig. 1. Map of the Cantal department, in the Auvergne region, showing the location of: A, the 1998 MSA survey; B, the Saint-Martin-sous-Vigouroux survey; C: the Puy Mary peak; D, the Plomb-du-Cantal peak; E: Saint Flour city.

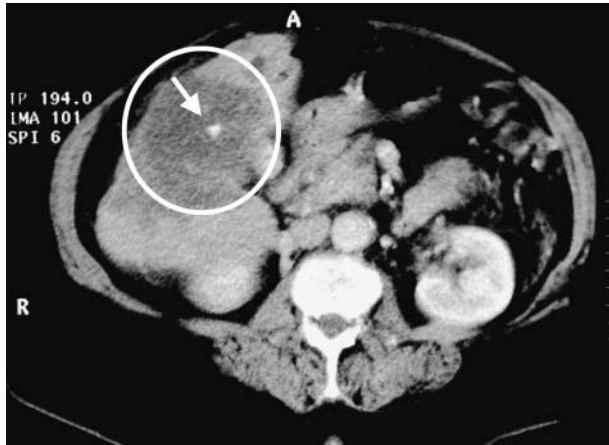


Fig. 2. Computed tomography of case's liver showing the parasitic mass (circle), along with a calcified central area (arrow).

hydatidosis infection was performed in the Department of Parasitology of the Toulouse University Hospital using ELISA (home-made test with *E. granulosus* soluble antigen from BioRad, Marnes-la-Coquette France), immunoelectrophoresis (IE, with the same antigen) and indirect haemagglutination (IHA, Fumouze Labs, Levallois Perret, France). The results were an optical density (OD) of 0.740 (normal value  $<0.400$ ) by ELISA, the presence of the band no. 5 by IE, and a negative IHA test. Such results were consistent with AE aetiology, and a confirmation by Western-blotting (LD Bio Products, Lyon, France) was required of the Laboratory of Parasitology, Besançon University Hospital. All bands reported in an *Echinococcus* sp. infection, namely 7 kDa, 16 kDa, 18 kDa, 26 kDa and 28 kDa, were detected. The observed pattern, which included two narrow bands at 16 kDa and 18 kDa, was very evocative of AE (Liance *et al.*, 2000). An inquiry to patient's doctor and to the Mayor of St Martin s/V. showed that the case was the spouse of a retired non-commissioned officer, and that the couple had never left the Billiez hamlet for the last 45 years, thus ascertaining the autochthonous origin of AE. The patient underwent an external biliary drain, associated with albendazole therapy ( $10 \text{ mg kg}^{-1}$  daily). Albendazole therapy was stopped after 1 month, due to a significant increase in liver enzyme values. The patient deceased 7 months later.

#### Survey in the sylvatic definitive host

For collecting fox carcasses, substantial assistance was provided by members of local hunting societies, managed by the official French 'lieutenants de louvèterie', volunteer officers in charge of the enforcement of hunting laws. From December 1999 to April 2000, several battues were organized in an ellipsoidal area (approximately  $20 \text{ km} \times 8 \text{ km}$ ), oriented SW from St Martin s/V. Eleven red foxes (*Vulpes vulpes*) were shot. It should be noted that these battues were not carried out specially for the AE survey, but were included in a plan for game preservation. A detailed technical form was filled out, especially indicating the killing location and the gender of the animals, then the foxes were promptly sent to a veterinary

office (maximum distance 20 km) where they were dissected. The intestines were cut after the tying of both pylorus and rectum, then stored at  $-18^\circ\text{C}$  in Auvergne until April 2000, when they were brought to the Department of Parasitology to be stored at  $-25^\circ\text{C}$ .

Moreover, from February 2000 to April 2000, 50 fox faecal deposits were collected in 18 different locations, situated roughly in a 25-km circle around St Martin s/V. The identification of fox origin was based upon the shape, size and location on the ground. The minimal distance between two sampling areas was approximately 2 km. After transfer in plastic tubes then into polyethylene bags, faeces were stored at  $-18^\circ\text{C}$ , then at  $-25^\circ\text{C}$  once brought to Toulouse. The sampling location was clearly identified on each tube.

Before processing and for safety enhancement, faeces and intestines were deep-frozen at  $-80^\circ\text{C}$  for 3 weeks (Eckert, 2003). Fox intestines were examined for adult worms at the Laboratory of Parasitology of the National Veterinary School in Toulouse (ENVT) using the scraping method, as described by Deplazes & Eckert (1996). Coproantigen ELISA assay was performed on scraping products and faeces suspensions, using the Chekit Echinotest™ (Dr Bommeli AG, Liebefeld-Bern, Switzerland), according to the manufacturer's instructions. The titre of a given sample was expressed as a percentage of the OD of a positive reference serum included in the kit. Samples displaying a titre  $>40\%$  were considered as positive, those showing a titre  $<30\%$  were considered negative, and the grey zone ranged from 30 to 40%.

#### Survey in the peridomestic definitive host

Between December 1999 and April 2000, 12 farm dogs, owned by cattle breeders in the St Martin s/V. commune, underwent faeces sampling by rectal touch, the product of which was stored at  $-18^\circ\text{C}$ . The coproantigen assay was carried out as described above. Four dogs displaying a positive or borderline ELISA result were subsequently treated with arecoline bromhydrate. The faeces were preserved in a 10% formalin solution, then examined by stereomicroscope at the Laboratory of Parasitology of the ENVT.

#### Survey in the rodent intermediate host

Volunteer inhabitants of St Martin s/V. trapped rodents in December 1999 and April 2000 around the case's home, and also in pastures within the commune area. Twenty-five small mammals were captured. After storage in Toulouse at  $-25^\circ\text{C}$ , they were sent to the University of Franche-Comté, Besançon, for identification and AE diagnosis.

#### Survey in humans

The work carried out was of the cluster survey type (Dabis *et al.*, 1992). Following the Mayor's call for participation, 85 volunteers from 35 families presented themselves at the St Martin s/V. city hall. For each subject, a demographic and epidemiological questionnaire was completed concerning identity, age, sex, place of residence, occupation, personal physician's address,

presence of a kitchen garden at home (fenced or not), ownership of dogs and/or cats, status of the pets (regularly dewormed or not), presence of rodents around the home, hunting, and consumption of wild dandelion (*Taraxacum dens leonis*) salads, a local dish in Auvergne. Minors less than 10 years old were not included in the survey. After informed oral consent, and after parent's agreement for minors over 10 years old, blood samples were taken to perform total and differential blood counts, as well as immunodiagnosis of cystic hydatidosis (see above), of fascioliasis by ELISA (home-made test with *Fasciola hepatica* soluble antigen from Bio-Rad, Marnes-la-Coquette France) and IHA (Fumouze Labs, Levallois Perret, France) and of toxocariasis by Western-blot (WB) and by specific anti-*Toxocara* IgE detection, both tests using home-made excretory–secretory antigen from *Toxocara canis* larvae. For the serodiagnosis of AE, ELISA Em2 Plus kit (Bordier Affinity Products, Crissier, Switzerland) was used. According to the manufacturer's instructions, any serum displaying an OD  $\geq 0.300$  was considered as positive, and the grey zone ranged from OD 0.150 to OD 0.299. A confirmation test by WB (LD Bio, Lyon, France) was performed for any subject displaying an OD value  $\geq 0.075$ , and also for those in whom a blood eosinophilia  $> 400$  cells  $\mu\text{l}^{-1}$  was found, or for those having a result of ELISA hydatidosis  $\geq \text{OD } 0.100$ , whatever the result of ELISA Em2 Plus.

## Results

In foxes, after intestine scraping, no *E. multilocularis* worms were found by stereomicroscope examination of the products. Using the Chekit Echinotest™, one sample was found positive (titre 45%), but this animal harboured a large tapeworm. No results were in the grey zone. Of 18 batches of faeces, each one corresponding to a different place of collection, only one batch showed two positive samples (titres 43% and 76.5%, respectively) out of four, and another batch two border-line results (titres 31% and 36%, respectively) out of 13.

Of 12 farm dogs, two were found positive (titres 42% and 78%, respectively) and two had borderline results (titres 37% and 39%, respectively). All these dogs came from different farms. The 42% (positive) and the 39% (borderline) results were from dogs said to be regularly dewormed with nitroscanate, according to owners' statements. However, after arecoline purgation, the microscopy examination of these four faecal specimens failed to find either adult *E. multilocularis* or other worms.

The 25 trapped small mammals consisted of 19 *A. terrestris*, two *Clethrionomys glareolus*, two *Apodemus* sp., one *Rattus norvegicus* and one *Talpa europea*. Twenty-three specimens (*A. terrestris*, *C. glareolus* and *Apodemus* sp.) could be considered as potential hosts for *E. multilocularis* (Houin *et al.*, 1980; Pétavy *et al.*, 1984, 1990). After necropsy, one *A. terrestris* female showed a 1-mm whitish spot on the liver. Pathological examination reached a conclusion of a degenerated parasitic structure consistent with an *E. multilocularis* metacystode.

Of 85 volunteers, no positive serology for AE or for hydatidosis or fascioliasis was found. Forty subjects had a positive WB result for toxocariasis, along with the

presence of specific anti-*Toxocara* IgE for 17 (range 2–3223 *Toxocara* Units  $\text{l}^{-1}$ ).

Only the case's husband displayed laboratory results consistent with a low-grade AE infection. The ELISA Em2 Plus result was OD 0.157, and WB showed a 7 kDA band. Immunodiagnosis of hydatidosis was negative (ELISA OD 0.037; IHA  $< 80$ ; IEP negative), as was that of both fascioliasis and toxocariasis. Eosinophil count was 200 cells  $\mu\text{l}^{-1}$ , but the dosage of serum total IgE was elevated at 801 kUI  $\text{l}^{-1}$  (normal value  $< 150$ ), along with a negative detection of IgE specific for the most common aeroallergens. A cross-reaction due to a tissue-dwelling helminth infection other than AE appeared therefore quite unlikely. In spite of a request made by his personal physician, the patient refused any further laboratory (liver enzymes) or radiology investigation. Fifteen volunteers had an ELISA Em2 Plus result of between OD 0.075 and 0.134, and three subjects displayed an ELISA hydatidosis result  $\geq \text{OD } 0.100$  along with an Em2 Plus result  $< \text{OD } 0.075$ . Confirmation test by Western-blotting was strictly negative (no bands observed) for these 18 subjects. Moreover, ten subjects had a blood eosinophil count  $\geq 400$  cells  $\mu\text{l}^{-1}$  (range 400–600), which could be explained by a toxocaral infection in four of the cases. For the six others, results of the conventional immunodiagnosis of both AE and hydatidosis were strictly negative. All ten sera were tested for AE by WB, which also displayed a negative result (no bands).

## Discussion

Both direct examination and detection of coproantigen in scraping products from fox intestines did not reveal the presence of *E. multilocularis* adult worms. The only putatively positive result (titre 45%) was a possible cross-reaction due to the presence of a large tapeworm (Eckert, 2003). The results from the coproantigen ELISA assay should be cautiously taken into account since no published estimation of Chekit Echinotest™ specificity is currently available.

The results of the coproantigen test on farm dogs were more interesting. After arecoline therapy, stereomicroscope examination of the stools from two positive animals did not reveal any adult nematode or cestode worms, which could have cross-reacted in the coproantigen assay. These two dogs (12.5%) could therefore harbour a light *E. multilocularis* burden.

Only one of 19 voles (5.26%, 95% CI 0.1–26.2) was found positive after necropsy. This rate is similar to those previously reported (range 0.0–4.65%) in the north-east part of the Cantal department (Pétavy & Deblock, 1983). Since it has been demonstrated that high prevalence rates are observed locally (Delattre *et al.*, 1988; Gottstein *et al.*, 2001; Giraudoux *et al.*, 2002) it would have been interesting to focus on rodents of the high pasture where the case usually gathered, for four decades, dandelions for salads. Unfortunately, due to the harsh winter climatic conditions existing on the Cantal volcano slopes, the site was not accessible.

The survey in 85 human volunteers failed to find any further AE positive subjects. Only the case's husband was suspected of having the disease, and could not be



included in the statistics concerning the St Martin s/V. population, since this possible contamination was obviously due to the cluster effect. Epidemiological data indicated, however, that risk factors for transmission of any helminth saprozoonose were present (table 1) namely a high-rate of dewormed animals among the peridomestic potential definitive hosts, along with the frequent presence of rodents around the houses, a large proportion of unfenced kitchen-gardens, and roughly two subjects out of three having the local habit of eating wild dandelion salads. Toxocariasis, which is also a soil-transmitted helminth zoonosis, was detected by WB in 40 volunteers out of 85 (47.05%), of whom four (4.7%) had an active infection by the time of blood sampling, thus underlining the permanent transmission of another geohelminth infection in the studied human population.

These risk factors linked to human behaviour might be amplified by the association with a high local density of water voles (Viel *et al.*, 1999). This point was inferred from the results of the epidemiological questionnaire (table 1) showing that the presence of rodents around houses was observed by 68.6% of the families. During the winter 1998–1999, the water vole density in the St Martin s/V. commune was <100 voles ha<sup>-1</sup>, but the rodent population has been increasing throughout the Cantal department, according to data from the local Office of the French Ministry of Agriculture. In the St Martin s/V. commune, the wooded areas (mainly beeches) cover approximately 20% of the ground surface, the remaining surface being mainly grasslands. Combined with the local orography and meteorological characteristics, these factors are highly favourable for water vole outbreaks and intensive transmission (Gilot *et al.*, 1988; Delattre *et al.*, 1992; Viel *et al.*, 1999).

This preliminary survey concerning AE, which was carried out at the southern limit of the parasite distribution in France, failed to clearly demonstrate the presence of *E. multilocularis* in the few definitive hosts which were investigated, but an AE-like lesion was detected in one intermediate host. The EurEchinoreg database indicated that between 1989 and 1993, three cases of human AE were diagnosed in the Saint Flour administrative district which covers grossly the lower right quadrant of the Cantal department. Added to the case from this study, they suggest that a slow extension of AE to the south, from the northern Cantal endemic area,

could be in progress. As a consequence, local administrative, sanitary and political authorities must be informed of these findings, in order to inform the population of this district of a potential risk of transmission. Regular deworming of pets with praziquantel, and a change in local culinary habits are safety measures that district residents should be informed of. Further large epidemiological surveys in southern Cantal, to refine the data obtained by the present study, are also necessary.

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Table 1. Demographic and epidemiological data from 85 volunteers representing 35 families from Saint-Martin-sous-Vigouroux, Cantal, France.

Mean age (years)	48
Age range	11–86
Farmer or cattle breeder	49.4%
Kitchen garden around home	89.4%
Fenced kitchen garden	68.2%
Dog ownership	83.5%
Compliance to dog deworming	76.5%
Cat ownership	58.8%
Compliance to cat deworming	22.3%
Hunting	16.5%
Consumption of wild dandelion salads	67.1%
Presence of rodents around home (% of families)	68.2%

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