



**HAL**  
open science

## **Towards delimitation of the *Echinococcus multilocularis* parasite's southernmost range in France**

Gérald Umhang, Céline Richomme, Christophe Caillot, Vanessa Bastid, Jean-Marc Boucher, Jean-Louis Moyen, Corinne Novella, Bruno Richoux, Bernard Davoust, Franck Boué

► **To cite this version:**

Gérald Umhang, Céline Richomme, Christophe Caillot, Vanessa Bastid, Jean-Marc Boucher, et al.. Towards delimitation of the *Echinococcus multilocularis* parasite's southernmost range in France. *Veterinary Parasitology: Regional Studies and Reports*, 2022, 30, pp.100724. 10.1016/j.vprsr.2022.100724 . anses-03627080

**HAL Id: anses-03627080**

**<https://anses.hal.science/anses-03627080>**

Submitted on 1 Apr 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Short communication

2

3 Towards delimitation of the *Echinococcus multilocularis* parasite's southernmost range in France

4

5 Gérald Umhang<sup>1\*</sup>, Céline Richomme<sup>1</sup>, Christophe Caillot<sup>1</sup>, Vanessa Bastid<sup>1</sup>, Jean-Marc Boucher<sup>1</sup>, Jean-  
6 Louis Moyen<sup>2</sup>, Corinne Novella<sup>3</sup>, Bruno Richoux<sup>4</sup>, Bernard Davoust<sup>5,6</sup>, Franck Boué<sup>1</sup>.

7 **Affiliations:**

8 <sup>1</sup>Anses, Rabies and Wildlife Laboratory, National Reference Laboratory for *Echinococcus* spp.,  
9 Malzéville, France

10 <sup>2</sup>Laboratoire Départemental d'Analyse et de Recherche de la Dordogne (LDAR24), 24660 Coulounieix-  
11 Chamiers, France

12 <sup>3</sup>Laboratoire des Pyrénées et des Landes (LPL)

13 <sup>4</sup>Laboratoire départemental d'analyse de la Charente (LDA16)

14 <sup>5</sup>Animal Epidemiology Expert Group, French Military Health Service, Tours, France

15 <sup>6</sup>IHU Méditerranée Infection, Marseille, France

16

17 \*Corresponding author at: ANSES, Rabies and Wildlife Laboratory, National Reference Laboratory for  
18 *Echinococcus* spp., Malzéville, France

19 E-mail address: [gerald.umhang@anses.fr](mailto:gerald.umhang@anses.fr) (G. Umhang)

20

21

22 **Abstract:**

23 Alveolar echinococcosis is a severe, potentially fatal, parasitic disease caused by ingestion of  
24 microscopic eggs of *Echinococcus multilocularis*. The lifecycle of the parasite is essentially sylvatic, and  
25 based on a prey-predator relationship between red foxes and small rodents. A westward expansion  
26 from the eastern historical focus has been reported in France, though the parasite has also been  
27 detected in the southern Alps. While the focus in the Auvergne region (central France) was described  
28 in the 1980s, the southern delimitation of the actual endemic area, especially in the south, was  
29 unknown in the absence of dedicated surveys. Red fox samples were collected from 2013 to 2020 in  
30 the framework of other transversal epidemiological studies in five sampling areas from southwestern  
31 and southeastern France. One hundred and seven intestines were analysed by SSCT, and 221 faecal  
32 samples from intestines were analysed by copro-qPCR. None of the 328 foxes exhibited *E.*  
33 *multilocularis* worms or DNA. Although the presence of *E. multilocularis* cannot be totally excluded in  
34 the departments from the study areas, the sample size tested argues for an absence of the parasite in  
35 these studied areas, which is in accordance with the currently known endemic situation in France.  
36 These new data are helpful in determining the southernmost limit of *E. multilocularis* distribution in  
37 France. The warm, dry Mediterranean climate in the southeastern areas is less favourable to the  
38 transmission of *E. multilocularis* and especially to the survival of eggs in the environment than the  
39 climate in the French Alps or Liguria (Italy) climate where the parasite is present. The intermediate  
40 area between the southwestern study areas and the historical focus of Auvergne, which is separated  
41 by around 150 km, will be investigated in the coming years. Moreover, an ongoing national surveillance  
42 programme on *E. multilocularis* in foxes is targeting French départements along the edge of the known  
43 endemic area both in the southeast and southwest. The data produced will supplement the results of  
44 this study, thus greatly helping to define the current distribution of *E. multilocularis* in France and to  
45 target prevention measures to reduce human exposure.

46 **Keywords:** *Echinococcus multilocularis*, surveillance, France, red fox

47 1. Introduction

48 Alveolar echinococcosis (AE) is a severe parasitic disease fatal to patients if not appropriately  
49 diagnosed and treated (Torgerson et al., 2010). The infection is due to oral ingestion of eggs of the  
50 tapeworm *Echinococcus multilocularis*, which reaches the liver from the intestines via portal blood  
51 flow. The larval stage gradually proliferates and infiltrates the liver parenchyma, thus destroying the  
52 surrounding host tissue, and may also affect other organs by infiltration or metastasis (Eckert et al.,  
53 2001). After a long asymptomatic period of up to 15 years, human AE may be diagnosed in patients  
54 due to a manifestation of symptoms or, increasingly often, coincidentally. The primary lifecycle of the  
55 parasite in Europe involves red foxes (*Vulpes vulpes*) and small rodents mainly from the *Arvicolinae*  
56 sub-family as respectively definitive and intermediate hosts. Worms in fox intestines release the  
57 parasite's eggs into the environment through faeces. Rodents develop alveolar echinococcosis after  
58 ingesting the eggs. The life cycle is completed when foxes then predate infected rodents. While the  
59 parasite was initially identified in only a few western European countries at the end of the 19<sup>th</sup> century,  
60 its current range now includes 21 European countries (Oksanen et al., 2016) due to expansion through  
61 the dispersal movement of foxes from the historical focus (Knapp et al., 2009; Umhang et al., 2021a).  
62 In France, the first focus was described at the end of the 19th century in a few departments (French  
63 administrative units corresponding to NUTS3 level in European Union NUTS nomenclature) in  
64 northeastern France (Vuitton et al., 2015), then a second one was identified more recently in the  
65 Auvergne region (Figure 1), central France (Petavy and Deblock, 1980; Petavy and Deblock, 1983). A  
66 westward expansion from the historical eastern focus has since been reported (Combes et al., 2012)  
67 though assumed to be at least several decades old (Umhang et al., 2014). According to the absence of  
68 the parasite in the westernmost department (Morbihan) and detection of infected foxes only in the  
69 eastern part of the bordering Brittany (Bretagne administrative region, east of Ille-et-Vilaine  
70 department), the northwestern delimitation of the endemic areas to be currently roughly positioned  
71 at the east of Brittany (Combes et al., 2013; Combes et al., 2012). More recently, a southward  
72 expansion along the Alps was determined by the detection of the parasite's DNA in three fox faecal

73 samples in the Hautes-Alpes department (Umhang et al., 2016) and later of infected *Arvicola terrestris*  
74 (Umhang et al., 2021b). Furthermore, the parasite's DNA has been identified even further south, near  
75 the French-Italian border, in both wolf and dog faeces (Massolo et al., 2018). No surveys have yet  
76 investigated southwestern France, and the parasite has not been reported in Spain or Portugal  
77 (Oksanen et al., 2016). However, the parasite's current geographical distribution in southern France is  
78 thought to lie further south than the range currently indicated by reported cases. The aim of this study  
79 was to use convenience sampling of red fox samples in order to explore the parasite's potential  
80 presence far beyond the known endemic areas in southeastern and southwestern France.

81

## 82 2. Materials and Methods

83 Samples were collected from 2013 to 2020 in the framework of other transversal epidemiological  
84 studies in five sampling areas (Figure 1). Two areas, A and B, are military camps located in the Var and  
85 Bouches-du-Rhône departments (southeastern France, 350 km<sup>2</sup> and 15 km<sup>2</sup> respectively) where foxes  
86 (n=77) were sampled for investigations from 2013 to 2020 on vector-borne pathogens (Medkour et al.,  
87 2020). The three other areas are located in Charente (C: 539 km<sup>2</sup>), Dordogne (D: 525 km<sup>2</sup>) and Landes  
88 (E: 504 km<sup>2</sup>) departments (southwestern France) where foxes were sampled for research on  
89 *Mycobacterium bovis* from 2017 to 2020 (n=30, n=147 and n=74 respectively) (Richomme et al., 2020).  
90 Foxes were trapped throughout the years for areas A and B and between October and March for areas  
91 C, D and E. All fox carcass used in the present study were obtained in agreement with national  
92 regulations. As the study did not involve invasive procedures on live animals, no ethical approval was  
93 necessary. The fox intestines from areas A, B and C were collected during the necropsy and frozen at -  
94 20°C. For foxes from areas D and E, faecal samples were taken from the rectum and frozen at -20°C.  
95 All the intestinal and faecal samples were frozen at -80°C for a week prior to laboratory analyses in  
96 order to prevent any risk of zoonotic infection.

97 The intestines from areas A, B and C were analysed according to the SSCT (Segmental Sedimentation  
98 and Counting Technique) method (Umhang et al., 2011). Briefly, intestine of red foxes were divided in  
99 five equal parts and the first (from anterior to posterior parts) and fourth were scrapped to remove  
100 the intestinal content which is diluted with water to look for the presence of *E. multilocularis* worms  
101 with a binocular. This method is very sensitive (98.4%) regarding the intestinal reference method SCT  
102 (Sedimentation and Counting Technique) which is considered the gold standard. For foxes from area  
103 D, DNA was extracted from 500 mg of faeces with a KingFisher Flex automated extraction system  
104 (Thermo Scientific) according to the manufacturer's instructions after supplemental mechanical lysis  
105 using an LSI MagVet Universal Isolation Kit (Life Technologies). Five samples of fox faecal samples  
106 previously identified as naturally infected by *E. multilocularis* were used as a positive control for this  
107 automated DNA extraction step. For foxes from area E, the QIAamp Fast DNA stool mini kit (Qiagen)  
108 was used, again according to the manufacturer's recommendations. All the copro-DNA samples were  
109 submitted for *E. multilocularis* detection by a real-time PCR based on the amplification of a part  
110 of the mitochondrial *rrnL* gene including an internal control (Knapp et al., 2016). A final qPCR volume  
111 of 20µL was used, containing 5µL of DNA, 10µL of Master Mix Maxima Probe, 50 copies of the internal  
112 control plasmid, 1.5µM of primers for *E. multilocularis* and internal control, 0.2µM and 0.1µM of *E.*  
113 *multilocularis* and internal control probe, respectively. The qPCRs were performed in duplicate and run  
114 on a Mx3005P thermocycler (Agilent) was used with a program that consists of 10 min at 95°C and 45  
115 cycles of 15 s at 95°C and 60 s at 60°C. In the event of inhibition, the DNA samples were diluted to  
116 1/10th before being re-tested.

117

### 118 3. Results and discussion

119 Previous knowledge on the distribution of *E. multilocularis* in France was mainly based on the parasite's  
120 detection in foxes with a sampling effort ranging from about 1 to 2 foxes per 100 km<sup>2</sup> in each of the  
121 departments investigated (Combes et al. 2012, Combes et al. 2013, Umhang et al. 2016) (Figure 1). In

122 the present study, the sampling areas were smaller due to the use of convenient sampling, but the  
123 sampling effort was much higher, ranging from 5 to 400 foxes per 100 km<sup>2</sup> in the five sampling areas  
124 (Table 1). None of the 328 foxes analysed had either *E. multilocularis* worms or their DNA. Considering  
125 the density of collected foxes, this result argues in favour of an absence of the parasite in the studied  
126 areas, even if the presence of *E. multilocularis* cannot be totally excluded from a statistical point of  
127 view (upper limit of the confidence interval at 95% of the prevalence: 2.5% in area D in the  
128 southwestern, and 4.7% in the southeastern areas). These new data, which show that no parasites  
129 were detected, are in accordance with previous knowledge on the endemic situation in France and are  
130 helpful in delimiting the southernmost range of the geographical distribution of *E. multilocularis* in  
131 France.

132 In 2016, *E. multilocularis* DNA was detected from fox faecal samples in the Hautes-Alpes (Umhang et  
133 al., 2016) in southeastern France, approximately 100 kilometres north of area A (Var). According to  
134 EmsB microsatellite analyses obtained from infected *Arvicola terrestris* specimens in the same Alpine  
135 area (Umhang et al., 2021b), the presence of the parasite there is thought to be the result of an  
136 expansion from the northeastern historical focus. Furthermore, the parasite's DNA was also detected  
137 in wolf and dog faeces in Italy (Massolo et al., 2018), suggesting the cestode's presence in the French-  
138 Italian border region (Alpes-Maritimes department). The southernmost positive wolf faecal sample  
139 from Italy were from the Province of Imperia (western part of the Liguria region), only 110 km  
140 northeast of Area A, found negative for the parasite in the current study. This could be explained by  
141 the warm, dry Mediterranean climate of area A, which is less favourable to the transmission of *E.*  
142 *multilocularis* and especially to the survival of eggs in the environment than the climate of the French  
143 Alps or Liguria.

144 The most southwestern *E. multilocularis* reports concern the historical focus of the Auvergne region  
145 (central France) (Figure 1). The first reports were made in the 1980s (Petavy and Deblock, 1980; Petavy  
146 and Deblock, 1983). By the early 2000s, the prevalence in foxes was estimated to stand at 9% (95% CI:

147 5%-17%) in the Cantal department (southern Auvergne) (Combes et al., 2012). An ongoing survey in  
148 this region aims to assess the presence of the parasite among foxes and its origin using the EmsB  
149 microsatellite approach. The areas of southwestern France investigated in the present study (areas C,  
150 D and E) are 150 km west of the westernmost cases reported in Auvergne. This intermediate area will  
151 be investigated in the coming years as part of the above-mentioned survey in Auvergne. Moreover, a  
152 national surveillance programme on *E. multilocularis* in foxes is under way and will target departments  
153 along the edge of the known endemic area, both in southeastern and western France. The data thus  
154 produced will supplement our own study results, thereby advancing efforts to define the actual  
155 distribution of *E. multilocularis* in France today, and to target preventive measures to reduce human  
156 exposure.

157

158

#### 159 **Declaration of Competing Interests**

160 The authors declare no competing interests in association with this study.

161

#### 162 **Acknowledgements**

163 The authors would like to thank local hunting federations, trappers and pest control officers for  
164 collecting foxes.

165

#### 166 **Funding**

167 This research did not receive any specific grant from funding agencies in the public, commercial, or  
168 not-for-profit sectors.



**Table 1:** Prevalence and confidence interval of *E. multilocularis* in the five study areas in southern France according to sample type and analysis method.

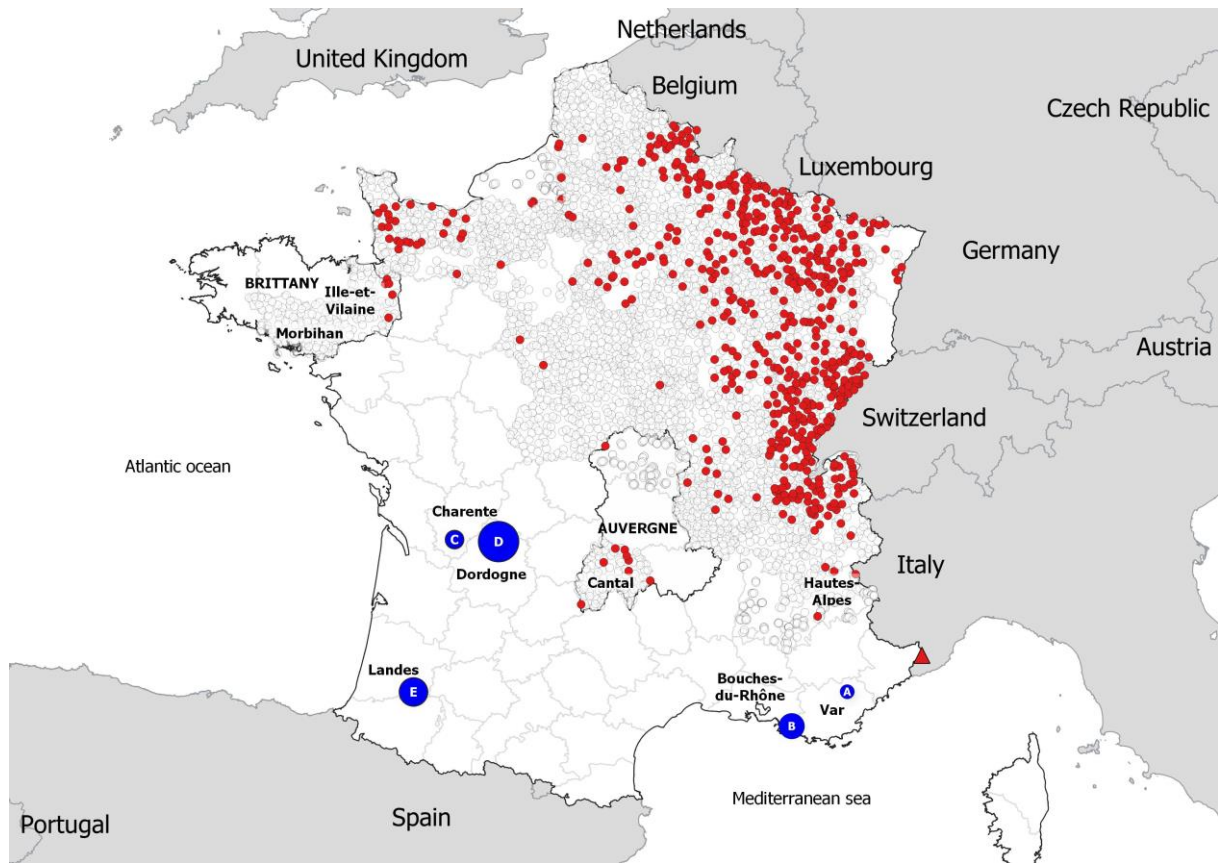
Areas	Departments	Nb of samples	Type of samples	Diagnostic method	Prevalence [95% confidence interval in %]	Density of fox sampled/100 km <sup>2</sup>
A	Var	17	intestines	SSCT	0 [0-19.5]	5
B	Bouches-du-Rhône	60	intestines	SSCT	0 [0-6.0]	400
C	Charente	30	intestines	SSCT	0 [0-11.6]	6
D	Dordogne	147	faeces	copro-qPCR	0 [0-2.5]	28
E	Landes	74	faeces	copro-qPCR	0 [0-4.9]	15

**Table 2:** Helminths found at a macroscopic level during the SSCT analyses (which focus on the first and fourth segments of intestine when divided in five equal parts) and rate of infection in foxes in three areas of the study where this research was implemented. The specific determination of nematodes has not been performed.

Areas	Departments	Number of fox analysed	Number of fox found infected (rate of infection)			
			<i>Mesocestoides sp.</i>	<i>Taenia sp.</i>	<i>Amoebotaenia sp.</i>	Nematoda (unregards the species)
A	Var	17	7 (41%)	6 (35%)	0	1 (6%)
B	Bouches-du-Rhône	60	26 (43%)	26 (43%)	1 (2%)	16 (27%)
C	Charente	30	20 (67%)	8 (27%)	2 (7%)	3 (10%)

172

173



174

175 **Figure 1:** The five study areas are indicated by blue circles; the sample size in each area is proportional  
 176 to the diameter of the circle and previous data on the distribution of *E. multilocularis* among foxes in  
 177 France (red circle for individual positive fox, white circle for negative individual fox) and at the Italian  
 178 border (red triangle for positive individual fox or dog) among dogs and wolves. The names of the French  
 179 regions (in upper case) and departments (in lower case) mentioned in the article are indicated on the  
 180 map.

181

182

183

184 **References:**

- 185 Combes, B., Comte, S., Raton, V., Raoul, F., Boué, F., Umhang, G., Favier, S., Dunoyer, C., Woronoff-  
186 Rehn, N., Giraudoux, P., 2013. Expansion géographique du parasite *Echinococcus*  
187 *multilocularis* chez le renard en France. *Bulletin Épidémiologique Santé Animale -*  
188 *Alimentation*, 16-18.
- 189 Combes, B., Comte, S., Raton, V., Raoul, F., Boué, F., Umhang, G., Favier, S., Dunoyer, C., Woronoff, N.,  
190 Giraudoux, P., 2012. Westward spread of *Echinococcus multilocularis* in foxes, France, 2005–  
191 2010. *Emerg. Infect. Dis.* 18, 2059-2062.
- 192 Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z.S., 2001. WHO/OIE Manual on echinococcosis in  
193 humans and animals : a public health problem of global concern. O.I.E. - O.M.S., Paris, 265 p p.
- 194 Knapp, J., Bart, J.M., Giraudoux, P., Glowatzki, M.L., Breyer, I., Raoul, F., Deplazes, P., Duscher, G.,  
195 Martinek, K., Dubinsky, P., Guislain, M.H., Cliquet, F., Romig, T., Malczewski, A., Gottstein, B.,  
196 Piarroux, R., 2009. Genetic diversity of the cestode *Echinococcus multilocularis* in red foxes at  
197 a continental scale in Europe. *PLoS Neglected Tropical Diseases* 3, e452.
- 198 Knapp, J., Umhang, G., Poulle, M.L., Millon, L., 2016. Development of a real-time PCR for a sensitive  
199 one-step copro-diagnosis allowing both the identification of carnivore feces and the detection  
200 of *Toxocara* spp. and *Echinococcus multilocularis*. *Appl. Environ. Microbiol.* 82, 2950-2958.
- 201 Massolo, A., Valli, D., Wassermann, M., Cavallero, S., D'Amelio, S., Meriggi, A., Torretta, E., Serafini, M.,  
202 Casulli, A., Zambon, L., Boni, C.B., Ori, M., Romig, T., Macchioni, F., 2018. Unexpected  
203 *Echinococcus multilocularis* infections in shepherd dogs and wolves in south-western Italian  
204 Alps: A new endemic area? *International Journal for Parasitology: Parasites and Wildlife* 7, 309-  
205 316.
- 206 Medkour, H., Laidoudi, Y., Marié, J.L., Fenollar, F., Davoust, B., Mediannikov, O., 2020. MOLECULAR  
207 INVESTIGATION OF VECTOR-BORNE PATHOGENS IN RED FOXES (*VULPES VULPES*) FROM  
208 SOUTHERN FRANCE. *J Wildl Dis* 56, 837-850.
- 209 Oksanen, A., Siles-Lucas, M., Karamon, J., Possenti, A., Conraths, F.J., Romig, T., Wysocki, P., Mannocci,  
210 A., Mipatrini, D., La Torre, G., Boufana, B., Casulli, A., 2016. The geographical distribution and  
211 prevalence of *Echinococcus multilocularis* in animals in the European Union and adjacent  
212 countries: a systematic review and meta-analysis. *Parasit Vectors* 9, 519.
- 213 Petavy, A.F., Deblock, S., 1980. [Helminths of the common fox (*Vulpes vulpes* L.) from the massif central  
214 (France) (author's transl)]. *Ann Parasitol Hum Comp* 55, 379-391.
- 215 Petavy, A.F., Deblock, S., 1983. Connaissance du foyer auvergnat d'échinococcose alvéolaire. *Ann.*  
216 *Parasitol. Hum. Comp.* 58, 439-453.
- 217 Richomme, C., Réveillaud, E., Moyen, J.L., Sabatier, P., De Cruz, K., Michelet, L., Boschioli, M.L., 2020.  
218 *Mycobacterium bovis* Infection in Red Foxes in Four Animal Tuberculosis Endemic Areas in  
219 France. *Microorganisms* 8.
- 220 Torgerson, P.R., Keller, K., Magnotta, M., Ragland, N., 2010. The Global Burden of Alveolar  
221 *Echinococcosis*. *PloS Neg. Trop. Dis.* 4, e722.
- 222 Umhang, G., Bastid, V., Avcioglu, H., Bagraade, G., Bujanić, M., Bjelić Čabrilo, O., Casulli, A., Dorny, P.,  
223 van der Giessen, J., Guven, E., Harna, J., Karamon, J., Kharchenko, V., Knapp, J., Kolarova, L.,  
224 Konyaev, S., Laurimaa, L., Losch, S., Miljević, M., Miterpakova, M., Moks, E., Romig, T., Saarma,  
225 U., Snabel, V., Sreter, T., Valdmann, H., Boué, F., 2021a. Unravelling the genetic diversity and  
226 relatedness of *Echinococcus multilocularis* isolates in Eurasia using the EmsB microsatellite  
227 nuclear marker. *Infect Genet Evol* 92, 104863.
- 228 Umhang, G., Comte, S., Hormaz, V., Boucher, J.M., Raton, V., Favier, S., Raoul, F., Giraudoux, P.,  
229 Combes, B., Boue, F., 2016. Retrospective analyses of fox feces by real-time PCR to identify  
230 new endemic areas of *Echinococcus multilocularis* in France. *Parasitology Research* 115, 4437-  
231 4441.
- 232 Umhang, G., Demerson, J.-M., Legras, L., Boucher, J.-M., Peytavin de Garam, C., Bastid, V., Vannard, E.,  
233 Pinot, A., Giraudoux, P., Boué, F., 2021b. Rodent control programmes can integrate

234 Echinococcus multilocularis surveillance by facilitating parasite genotyping: the case of  
235 *Arvicola terrestris* voles screening in France. Parasitology Research in press.  
236 Umhang, G., Knapp, J., Hormaz, V., Raoul, F., Boue, F., 2014. Using the genetics of *Echinococcus*  
237 *multilocularis* to trace the history of expansion from an endemic area. Infection, Genetics and  
238 Evolution 22, 142-149.  
239 Umhang, G., Woronoff-Rhen, N., Combes, B., Boue, F., 2011. Segmental sedimentation and counting  
240 technique (SSCT): an adaptable method for qualitative diagnosis of *Echinococcus multilocularis*  
241 in fox intestines. Exp. Parasitol. 128, 57-60.  
242 Vuitton, D.A., Demonmerot, F., Knapp, J., Richou, C., Grenouillet, F., Chauchet, A., Vuitton, L., Bresson-  
243 Hadni, S., Millon, L., 2015. Clinical epidemiology of human AE in Europe. Vet Parasitol 213, 110-  
244 120.