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1 Short communication

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

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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 19th 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 km² and 15 km² 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 km²), Dordogne (D: 525 km²) and Landes
88 (E: 504 km²) 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 km² 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 km² 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

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

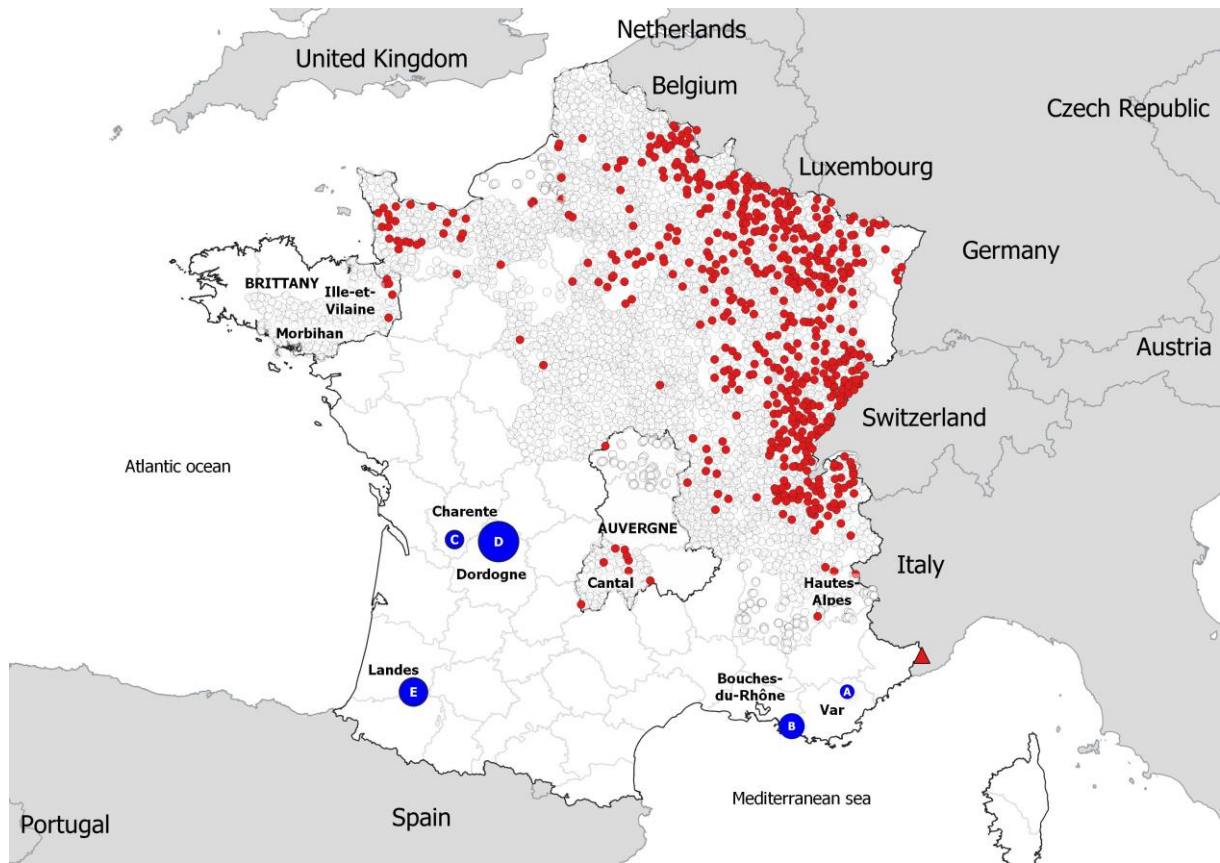
Areas	Departments	Nb of samples	Type of samples	Diagnostic method	Prevalence [95% confidence interval in %]	Density of fox sampled/100 km ²
A	Var	17	intestines	SSCT	0 [0-19.5]	5
B	Bouches-du-Rhône	60	intestines	SSCT	0 [0-6.0]	400
C	Charente	30	intestines	SSCT	0 [0-11.6]	6
D	Dordogne	147	faeces	copro-qPCR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

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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.

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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 Epidémiologique 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.