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1 **Detection of *Echinococcus* spp. and other taeniid species in lettuces and berries: two**
2 **international multicenter studies from the MEmE project**

3
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52

53 **Abstract:**

54 Cystic and alveolar echinococcosis are severe zoonotic diseases characterized by long
55 asymptomatic periods lasting months or years. Viable *Echinococcus* eggs released into the
56 environment through the feces of canids can infect humans through accidental ingestion via
57 hand-to-mouth contact or consumption of contaminated food or water. Both *Echinococcus*
58 *multilocularis* and *Echinococcus granulosus sensu lato* are considered as foodborne parasites.
59 However, when considering possible pathways of human infection, it appears that food and
60 water-borne related variables do not significantly increase the risk of infection. Providing
61 evidence-based data for the presence of DNA and, potentially, eggs in fresh produce is crucial
62 in understanding foodborne transmission of *Echinococcus* spp. to humans. Two multicenter and
63 multicountry studies were conducted within the One Health EJP framework to estimate the
64 proportion of lettuces and berries contaminated by *E. multilocularis*, *E. granulosus s.l.*, and
65 other taeniid DNAs from 12 European countries, Tunisia and Pakistan. A total of 1,117 lettuces,
66 300 strawberries and 130 blueberries samples were collected and analyzed by washing,
67 sequential sieving and real-time PCRs. *Echinococcus multilocularis* DNA was detected in 1.2%
68 (7/570) of samples tested from the seven European endemic areas and in 2% (2/100) from
69 Pakistan. In the five European endemic countries for *E. granulosus s.l.*, *E. granulosus sensu*
70 *stricto* DNA was identified in 1.3% of lettuces (9/695). The proportion of lettuces contaminated
71 by *E. granulosus sensu stricto* DNA was 12% (9/75) in Tunisia and 4% (4/100) in Pakistan.
72 Regarding berries, *E. multilocularis* DNA was detected in 5.4% of strawberries (n=11/202),
73 7.3% of blueberries (6/82) from the seven European endemic countries and 56% of blueberries
74 (14/25) from Pakistan. High contamination rates of *E. granulosus s.s* were found outside of
75 Europe, with 12.0% in blueberries (3/25) from Pakistan and 81.3%. DNA in strawberries

76 (13/16) from Tunisia. The total contamination rate of all taeniid species DNA in lettuces (5.3%;
77 59/1,117) and berries (12.1%; 58/480) suggests that the transfer of taeniid eggs from carnivore
78 feces to food is not uncommon. Although we assume that eggs are the source of the DNA
79 detected in this study, the viability of such eggs is unknown. The detection of *Echinococcus*
80 species in lettuces and berries suggests a potential risk of foodborne human infection. The
81 relative contribution of this risk remains to be estimated. Further studies on food and
82 environmental contamination are necessary to cover different epidemiological contexts and
83 social habits, leading to a better understanding of human infections by *Echinococcus* eggs.

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88 **Keywords:**

89 *Echinococcus multilocularis*, *Echinococcus granulosus sensu lato*, *Taenia* spp., foodborne
90 parasitic infections, environmental contamination, pathways of transmission, risk factors.

91

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93 **1. Introduction:**

94

95 The echinococcosis disease group is listed among the Neglected Tropical Diseases (NTDs) of
96 global public health importance prioritized by the World Health Organization (WHO) for their
97 control (WHO, 2024; Casulli and Tamarozzi, 2021). Both cystic (CE) and alveolar (AE)
98 echinococcosis are characterized by a long asymptomatic period lasting months or years. CE is
99 a chronic and disabling disease characterized by the development of parasitic cysts, mainly
100 located in the liver (70%), lungs (19%) or in uncommon localizations (11%) as bones, hearth,
101 muscles and central nervous system (Casulli, Pane, et al., 2023). The concentric growth of the
102 cysts may have an effect of compression on neighboring organs and tissues, which sometimes
103 can compromise their function. The mortality rate for CE is estimated around 2–4% but can be
104 higher if medical treatment and care are inadequate (Brunetti et al., 2010). An estimate of
105 around 65,000 human cases of CE was reported from 1997 to 2021 in 40 selected European
106 countries (Casulli, Abela-Ridder, et al., 2023). AE is generally considered to be a more serious
107 life-threatening disease than CE, as it is characterized by a tumor-like infiltrative parasitic tissue
108 (vesicles), mainly present in the liver with parasitic metastasis spreading in the neighboring
109 organs (Gottstein et al., 2017). AE is distributed in the Northern Hemisphere, while CE is
110 present worldwide. According to non-updated estimates, the annual global incidence of AE was
111 18,000 cases per year in 2010, of which 91% occurring in China (Torgerson et al., 2010). During
112 the period 2018-2022, official statistics from the 27 member states of the European Union have
113 reported an average of 690 unspecified echinococcosis human cases per year, including 338 CE
114 and 151 AE human cases (EFSA, 2022). Nevertheless, it should be kept in mind that
115 considerable underreporting is existing for both diseases with a heterogeneity in data reporting
116 among European countries (van der Giessen et al., 2021).

117 *Echinococcus multilocularis* and *Echinococcus granulosus sensu lato (s.l.)* are the causative
118 agents of AE and CE, respectively. The lifecycle of these two parasites is based on the trophic
119 relationship between canids as definitive hosts and ungulates or rodents as intermediate hosts.
120 In non-Arctic areas of Europe, the red fox (*Vulpes vulpes*) is the primary definitive host for *E.*
121 *multilocularis*, while in Asia, the dog is also heavily involved due to their frequent predation
122 on small rodents (Romig et al., 2017). The life cycle of *E. granulosus s.l.*, which is a complex
123 of cryptic species, is primarily domestic. The dog serves as the main definitive host, with
124 various livestock species acting as intermediate hosts, depending on the specific *E. granulosus*
125 species involved. *E. granulosus s.l.* is currently represented by five species (genotypes), namely
126 *E. granulosus sensu stricto (s.s.; G1 and G3)*, *E. equinus (G4)*, *E. ortleppi (G5)*, *E. canadensis*
127 *(G6/7 complex, G8 and G10)* and *E. felidis* (Romig et al., 2017; Vuitton et al., 2020). The latter
128 species has never been documented to infect humans and was only reported from Africa. The
129 other four species are present in Europe and worldwide with varying frequencies and
130 geographical distributions in humans (Alvarez Rojas et al., 2014; Casulli et al., 2022).
131 Compared to *E. granulosus s.l.*, the genetic diversity of *E. multilocularis* is considered relatively
132 low (Haag et al., 1997; Knapp et al., 2015). Four distinct clades of *E. multilocularis* are
133 currently recognized based on their geographical origin, as initially determined by
134 mitochondrial sequencing (Nakao et al., 2009) and further supported by EmsB nuclear
135 microsatellite analysis (Knapp et al., 2007; Umhang et al., 2021). Nevertheless, Asian-like
136 samples have been reported in Europe and European-like samples in North America (Karamon
137 et al., 2017; Santa et al., 2023).

138 Viable *Echinococcus* eggs can infect humans through accidental ingestion via hand-to-mouth
139 contact or consumption of contaminated food or water. The eggs are released into the
140 environment through the feces of canids (Alvarez Rojas et al., 2018; Kapel et al., 2006) and are
141 highly resistant to temperature variations under natural conditions. Eggs of *E. multilocularis*

142 can survive up to 240 days in autumn-winter and 78 days in summer, as reported in Germany
143 (Veit et al., 1995). Similarly, eggs of *E. granulosus s.l.* can survive up to 41 months, as
144 documented in Argentinian Patagonia (Sánchez Thevenet et al., 2019). Furthermore, taeniid
145 eggs can be dispersed passively over short and long distances by wind, rain, insects, or birds
146 (Benelli et al., 2021; Raymond and St Clair, 2023; Sánchez Thevenet et al., 2019), leading to
147 contamination of various environmental matrices. However, there is limited information
148 available on environmental contamination caused by these parasites in different matrices, such
149 as fox or dog fur, soil, water, vegetables, and fomites (Tamarozzi et al., 2020).

150 *Echinococcus multilocularis* and *E. granulosus s.l.* are both considered foodborne parasites,
151 ranking first and fourth in Europe and second and third in the world, respectively (Bouwknegt
152 et al., 2018; Devleeschauwer et al., 2017). Two systematic reviews and meta-analyses were
153 conducted on AE and CE using previously published case-control and cross-sectional studies
154 to identify potential risk factors (Conraths et al., 2017; Possenti et al., 2016). The results of both
155 studies suggested that, when considering possible pathways of human infection, food- and
156 water-borne related variables do not significantly increase the risk of infection with
157 *Echinococcus* spp. Another systematic review and meta-analysis from Torgerson et al. (2020),
158 used attributable fractions for the potential sources of CE and AE infections, instead of
159 considering risk factors. This study confirmed that food can be a source of human infections
160 for CE worldwide. This association was not statistically significant in regions with a high
161 incidence of AE, such as China, whereas it was significant in regions with a low incidence, such
162 as Europe (Torgerson et al., 2020). Only a few recent studies have provided data on the
163 detection of *E. multilocularis* DNA in raw vegetables, berries, and mushrooms. Large
164 differences in detection rates have been observed, ranging from 1.3% in lettuces (n=157)
165 purchased in Switzerland to 23.3% in forest fruits, vegetables, and mushrooms (n=103)
166 collected in Poland (Guggisberg et al., 2020; Lass et al., 2015a). Regarding *E. granulosus s.l.*,

167 M'Rad et al. (2020) found that 1.3% of vegetables (n=240), purchased from local markets in
168 Tunisia, were contaminated with *E. granulosus s.s.* eggs. Comparing the results between these
169 studies is challenging due to the use of different methods (for the enrichment of samples, DNA
170 extraction and molecular detection), with generally unevaluated performance or high detection
171 limits of these tests. Providing evidence-based data for the presence of DNA and potentially
172 eggs in fresh products is crucial in understanding foodborne transmission of *Echinococcus* spp.
173 to humans (Bouwknegt et al., 2018). This task is challenging since it requires a method to enrich
174 matrices and detect very low number of taeniid eggs in food items, combined with molecular
175 tests to identify the species involved. In this context, multicenter and large-scale studies,
176 utilizing the same protocols for the detection of the parasites is mandatory. Therefore, we took
177 advantage of the MEmE project (Multi-centre study on *Echinococcus multilocularis* and
178 *Echinococcus granulosus s.l.* in Europe: development and harmonisation of diagnostic methods
179 in the food chain) within the One Health EJP framework to conduct a multicenter and
180 multicountry studies. The aim of this study was to estimate the proportion of lettuces and berries
181 contaminated by *E. multilocularis*, *E. granulosus s.l.*, and others taeniid species DNAs on a
182 large geographical scale.

183

184 **2. Materials and methods**

185

186 **2.1. Evaluation of the limit of detection and robustness of the method**

187 A preliminary validation step of the method was conducted to confirm its sensitivity from
188 samples artificially spiked with *E. multilocularis* eggs. The method used in this study, to detect
189 DNA from taeniid eggs in both lettuces and berries, was previously described by Guggisberg
190 et al. (2020). The method involved three steps: 1) washing the samples, 2) sequential sieving of
191 the washing solution to obtain a pellet from the 20 μ m filter, and 3) DNA extraction (using here

192 DNeasy Blood & Tissue kit, Qiagen) from the pellet for molecular analysis to detect *E.*
193 *multilocularis* using a real-time PCR (Knapp et al., 2016). Lettuce heads and strawberry trays
194 were purchased from supermarkets. These two matrices, comprising 300g of lettuce leaves or
195 200g of strawberries, respectively, were spiked with a defined number of *E. multilocularis* eggs
196 before being washed, filtered, and molecularly identified. To validate the process, a preliminary
197 step consisting of spiking 10 and 5 lettuce samples with 10 and 5 eggs, respectively, was
198 conducted. For strawberries, 3 samples were spiked with 10 eggs and 2 samples with 5 eggs.
199 Subsequently, 3 replicates composed by 24 lettuce samples each and 3 replicates of 8 strawberry
200 samples each, were spiked with 3, 2 or 1 eggs each sample. The limit of detection (LOD) was
201 defined as the minimum number of eggs that can be detected in at least 23 out of the 24
202 replicates for lettuces or all 8 replicates for strawberries. All eggs used for spiking were obtained
203 from the feces of experimentally infected red foxes, as approved by the Anses/ENVA/UPEC
204 ethics committee and the French Ministry of Research (Apafis n°33541-202110081648536)
205 and inactivated at -80°C before use.

206 In summer 2020, a second preliminary validation step was carried out by sampling lettuces
207 collected from the field (i.e. local markets and private kitchen gardens) in north-eastern France
208 to test the robustness of the approach, before the implementation of the method in other
209 countries.

210

211 **2.2. Sampling of lettuces and berries**

212 The first multicenter study focused on lettuces, with sampling conducted during summer 2021
213 and involving 15 laboratories from 12 countries: Denmark (n=1), France (n=1), Germany (n=2),
214 Italy (n=3), Latvia (n=1), the Netherlands (n=1), Norway (n=1), Pakistan (n=1), Poland (n=1),
215 Portugal (n=1), Switzerland (n=1) and Tunisia (n=1). Each laboratory was asked to collect
216 between 50 and 100 heads of lettuce. Such collection should have been carried out in local

217 markets (4 samples from the same site), private kitchen gardens (2 from the same site) or from
218 supermarkets (4 from each shop). Additionally, six laboratories (from Campania and Sardinia
219 regions of Italy, Latvia, north of Germany and Switzerland) collected other green leafy
220 vegetables, mainly chard and parsley.

221 In summer 2022, a second multicenter study was organized, focusing on berries (mainly
222 strawberries and blueberries). In this study, blueberries will refer to low-growing Eurasian
223 blueberries (also named bilberries and corresponding to *Vaccinium angustifolium*, *V. boreale*,
224 *V. mytilloides* and *V. pallidum*) and not to North American blueberries (*V. darrowii* and *V.*
225 *corymbosum*) growing on taller, cultivated bushes. Twelve laboratories from 12 countries were
226 involved in this study on berries: Denmark, Estonia, Finland, France, Italy, Latvia, the
227 Netherlands, Pakistan, Poland, Portugal, Switzerland and Tunisia. Each laboratory was asked
228 to collect between 20 and 30 berry samples (100-200 g each). Each laboratory collected berry
229 samples from local markets, directly in the field and forest or with supplementation from
230 supermarkets. Only one sample per site was requested. The European countries were considered
231 to be endemic for *E. granulosus* or *E. multilocularis* according to EFSA and ECDC (EFSA,
232 2022).

233

234 **2.3. Analysis of field samples for the detection of *E. multilocularis*, *E. granulosus* s.l. and** 235 **others taeniid species**

236 The samples collected from the field in the different countries were processed as following: 1)
237 washing the samples with subsequent sedimentation of the rinsing liquid to obtain a pellet, 2)
238 sequential sieving of the pellet to obtain an enriched sediment from the last 20µm filter, and 3)
239 DNA extraction and molecular detection of the enriched pellet. All participants conducted step
240 1 (washing and sedimentation) to ensure a prompt analysis of fresh products within a few days
241 of purchase, thus avoiding any decomposition of the sample. Standard operating procedures for

242 the first step of washing and sedimentation of lettuces and berries were distributed to each
243 participant prior to sampling, to ensure a reproducibility of the method. The participants in the
244 two multicenter studies were instructed not to wash the food items before starting the analysis
245 (step 1). Steps 2 and 3, which involved filtration, DNA extraction, and molecular detection,
246 were carried out in a single laboratory (Anses LRFSN, Nancy, France) to ensure reproducibility
247 of the method and sensitive detection of parasite DNA. In Step 1, the washing process consisted
248 of mixing 500 ml of 0.02% Tween with up to 300 g of lettuce leaves or up to 250 g of berries.
249 This initial washing step used standard plastic bags (Guggisberg et al., 2020) provided by Anses
250 LRFSN (Nancy, France) to avoid potential bias with the use of different materials. External
251 lettuce leaves were not removed from the sample and were included in the washing process.
252 For berries, the vigorous manual washing step from the original protocol for lettuce was
253 replaced by an orbital shaker (2 sessions of 15 minutes each) to remove as much residue as
254 possible from the berries. The berries were also manually mixed between the two shaking
255 sessions to ensure that the entire surface of each berry was washed. After washing, the rinsing
256 liquid solution was transferred to another plastic bag for overnight sedimentation to obtain a
257 pellet. The pellet was stored at +4°C or -20°C before being transferred to France (Anses, Nancy
258 laboratory) for steps 2 and 3. All lettuce and berry samples collected in France were processed
259 continuously without the sedimentation step after washing that was necessary only for the other
260 partners.

261 During step 2, pools were created for sequential sieving by grouping two sediment samples
262 together if they originated from the same site. Most of the lettuce samples (93.5%) and a third
263 of berry samples (34.4%) were analyzed by pooling samples. If a positive molecular test was
264 obtained from a pooled samples, it was assumed that only one of the two samples was positive
265 when estimating the proportion of contamination. The samples were filtered sequentially

266 through 105 μm , 40 μm , and 20 μm mesh size. The final pellet deposited on the last 20 μm
267 mesh filter was rinsed with 0.02% Tween using a pipette.

268 In Step 3, following brief centrifugation to remove the supernatant, DNA was extracted from
269 the pellet using the conventional DNeasy Blood & Tissue kit (Qiagen) with an initial overnight
270 lysis step to optimize recovery of DNA from eggs. The real-time PCR method described by
271 Knapp et al. (2016) was used to detect *E. multilocularis* DNA. *Echinococcus granulosus s.l.*
272 DNA was detected using primers and probes for the specific detection of *E. granulosus s.s.*, *E.*
273 *canadensis* and *E. ortleppi* (Maksimov et al., 2020). Others taeniid species were identified
274 through conventional PCR (Trachsel et al., 2007) and confirmed by Sanger sequencing
275 performed by a private company (Eurofins GmbH, Germany).

276

277 **2.4. Statistics**

278 The detection rates of the different parasite species for each type of matrix or sources of supply
279 (kitchen garden, local markets, supermarkets or in the field or forest) were expressed as a
280 percentage with 95% confidence intervals. Comparisons based on matrix type were conducted
281 using either a chi-square test or a Fischer test if the sample size was too small.

282

283 **3. Results**

284 **3.1. Estimation of the limit of detection**

285 Based on the preliminary validation, the LOD for lettuces was estimated to be 3 eggs, as *E.*
286 *multilocularis* DNA was detected in 23 out of 24 lettuce head replicates (detection rate >95%)
287 (Supplementary Table 1). The detection rate of lettuce replicates spiked with 2 and 1 eggs was
288 75% (18/24) and 50% (12/24), respectively (Supplementary Table 1). The LOD for *E.*
289 *multilocularis* DNA in strawberry replicates was estimated to be 3 eggs based on the detection

290 of *E. multilocularis* DNA in all 8 samples spiked with 3 eggs. The detection rate for both 2 and
291 1 spiked eggs was 88% (7/8) (Supplementary Table 1).

292

293 **3.2. Detection of DNA of *E. granulosus s.l.*, *E. multilocularis* and other taeniid species in** 294 **lettuces and other leafy vegetables**

295 Out of the 1,117 collected lettuces, 570 were from *E. multilocularis* endemic areas of European
296 countries (Denmark, France, Germany, Latvia, the Netherlands, Poland and Switzerland)
297 (Table 1). *Echinococcus multilocularis* DNA was detected in 7 lettuce samples (1.2%; 7/570)
298 from four European countries (France, Denmark, Latvia and Switzerland) (Table 1). The
299 proportions of detection of *E. multilocularis* DNA in lettuces was comparable (around 1%) for
300 most of the countries where *E. multilocularis* was detected: Denmark (4%; 2/50), France (1.3%;
301 3/226), Latvia (1.6%; 1/62) and Switzerland (1.3%; 1/80). Two samples (2%) out of 100 from
302 Pakistan tested positive for *E. multilocularis* DNA. Furthermore, among the 55 non-lettuce
303 vegetable samples collected from endemic areas, one *E. multilocularis* positive sample (1.8%;
304 1/55) was found in a chard from northern Germany (Supplementary Table 2).

305 The overall contamination rate of *E. granulosus s.l.* among lettuce samples was 2% (22/1,117)
306 (Table 1). The contamination rate, if we only consider European countries, was 1% (9/942),
307 with variations between areas ranging from 0 to 6.5%. Except for one case of *E. canadensis*
308 species detected in Pakistan, the other findings were all *E. granulosus s.s.* An average rate of
309 3.4% (8/232) was obtained for the three Italian regions (Abruzzo, Campania and Sardinia) and
310 1.6% (1/62) in Latvia. In Tunisia, the rate of lettuce contamination was the highest at 12%
311 (9/75), while in Pakistan, the rate was 4% (4/100). No positive sample of *E. granulosus s.l.*
312 were detected in leafy vegetables other than lettuce (Supplementary Table 2).

313 The total proportion of other taeniid species detected in lettuces was 2.5% (28/1,117) (Table 1).

314 In Europe, the proportion of 1.7% (16/942) was lower compared to Tunisia (4.0%; 3/75) and

315 Pakistan (9.0%; 9/100). Fifteen samples of *Hydatigera* sp. (1.3%; 15/1,117), six of *Taenia*
316 *hydatigena* (0.5%; 6/1,117), and five of *Taenia saginata* (0.4%; 5/1,117), were identified, as
317 well as one of *Taenia multiceps* (0.1%). One of the three Tunisian lettuce samples with *T.*
318 *hydatigena*, also tested positive for *E. granulosus* s.s. In addition, the partial 12S rRNA gene
319 sequence obtained from a sample of lettuce from Norway did not allow differentiation between
320 *Taenia serialis* and *Taenia krabbei*. *Hydatigera* sp. was also detected in chard, basil, and sorrel
321 samples from Latvia and Norway (Supplementary Table 2). The DNA of the cestode species
322 *Atriotenia incisa* was identified in one lettuce sample from Latvia, as the Cest4-5 primers have
323 a wider amplification spectrum than just taeniid species (Trachsel et al., 2007).

324

325 **3.3. Detection of DNA of *E. granulosus* s.l., *E. multilocularis* and other taeniid species in** 326 **berries**

327 A total of 480 berry samples were collected, of which 300 were strawberries, 130 blueberries
328 and the remaining 50 samples were from eight other berry species: blackberries (n=13),
329 raspberries (n=21), red currants (n=5), blueberry bushes (n=4), red lingonberries (n=3), black
330 currants (n=2), white currants (n=1), Saskatoon berries (n=1). A total of 202 strawberry samples
331 and 82 blueberry samples were collected from European countries where *E. multilocularis* is
332 endemic (Denmark, Estonia, France, Latvia, the Netherlands, Poland, and Switzerland).
333 *Echinococcus multilocularis* DNA was detected in 5.4% (11/202) of the strawberry samples
334 (Table 2) and 7.3% (6/82) of the blueberry samples (Table 3) from these endemic European
335 countries. No *E. multilocularis* DNA was detected in strawberry or blueberry samples from
336 Poland or Switzerland. In the other European endemic countries, the level of contamination
337 ranged from 1.8% to 16.7%. The two Baltic countries had a high proportion of contamination,
338 with 13.3% (4/30) in both strawberries and blueberries from Latvia and 16.7% (5/30) in
339 strawberries from Estonia. Although no positive strawberry sample (0/54) was found in France,

340 *E. multilocularis* DNA was identified in 3.2% (1/31) of blueberries analysed. *E. multilocularis*
341 DNA was also detected in The Netherlands from blueberries (1/6) and strawberries (1/8) at
342 16.7% and 12.5%, respectively. In Denmark, *E. multilocularis* DNA was detected in 1.8%
343 (1/56) of strawberry samples. A high proportion (56%; 14/25) of blueberries from Pakistan
344 were also contaminated with *E. multilocularis* DNA. *Echinococcus multilocularis* DNA was
345 not found in other types of berries (Supplementary Table 3).

346 The species identification of *E. granulosus s.l.* DNA from berries was limited to *E. granulosus*
347 *s.s.* The overall contamination levels in this study were 5.3% (16/300) in strawberries and 3.1%
348 (4/130) in blueberries (Tables 2 and 3). *Echinococcus granulosus s.l.* DNA was only detected
349 in countries where the domestic life cycle of the parasite is known to be present, such as Italy,
350 Latvia, Pakistan, Poland, Portugal, and Tunisia. In Europe, the DNA was detected in 1.1%
351 (3/284) of strawberries and 1% (1/105) of blueberries. In European areas from the
352 Mediterranean basin (Portugal and Sardinia) and Eastern Europe (Estonia, Latvia, and Poland)
353 which are endemic for CE, the contamination rate of *E. granulosus s.s.* in strawberries (3/145)
354 and blueberries (1/48) was 2.1% for both. The level of contamination varied from 1.8% (1/55)
355 in Sardinia, to 5.9% (1/17) in Portugal and was highest in Poland with 7.7% (1/13) of
356 strawberries testing positive for *E. granulosus s.s.* DNA. *Echinococcus granulosus s.s.* DNA in
357 blueberries was detected in 3.3% (1/30) of samples from Latvia. Higher contamination levels
358 of *E. granulosus s.s.* DNA were found outside of Europe, with 12.0% (3/25) of in blueberries
359 from Pakistan and 81.3% (13/16) of strawberries from Tunisia. No *E. granulosus s.s.* DNA was
360 detected in other types of berries (Supplementary Table 3).

361 Other taeniid species, including *Hydatigera* sp. and *T. hydatigena*, were detected in strawberries
362 and blueberries during this study. Additionally, *Mesocestoides melesi* was detected in
363 blueberries from Latvia. The overall detection level of other taeniid species DNA was 1.3%
364 (4/300) and 1.5% (2/130) in strawberries and blueberries, respectively (Tables 2 and 3). In

365 Europe, only *Hydatigera* sp. was detected in strawberries at 1.1% (3/284). In Tunisia, *T.*
366 *hydatigena* was detected in 6.3% (1/16) of strawberries. Contamination of blueberries with both
367 *Hydatigera* sp. and *T. hydatigena* was observed at 8% (1/25; 1/25) in Pakistan. No taeniid
368 species DNA others than *Echinococcus* spp. were identified from other berries (Supplementary
369 Table 3).

370

371 **3.4. Differences in contamination according to type of samples and supplying sources**

372 As regards the supply sources of samples, the majority of lettuces (76.2%; 851/1,117) was
373 purchased from local markets, 19% (212/1,117) from local gardens and only 4.8% (54/1,117)
374 from supermarkets. *Echinococcus multilocularis* and *E. granulosus s.l.* DNAs were detected in
375 lettuces from all supply sources except *E. granulosus s.l.*, which was not detected in
376 supermarket lettuces. Strawberries were obtained from local markets (85%; 255/300), kitchen
377 gardens (10%; 30/300), supermarkets (4.7%; 14/300) and forest or field (0.3%; 1/300). The
378 blueberries were mainly collected from forests (58.5%; 76/130), 35.4% (46/130) from local
379 markets and 6.2% (8/130) from private gardens. *Echinococcus multilocularis* and *E. granulosus*
380 *s.s.* DNAs were only found in blueberries from private kitchen gardens and local markets, whilst
381 both parasite DNA was found in strawberries from all supply sources. Regarding the different
382 supply sources of lettuces in Europe (private kitchen gardens, local markets, supermarkets), no
383 significant difference were observed in the contamination by *E. multilocularis* ($p=0.057$) or by
384 *E. granulosus s.l.* ($p=0.157$). The same observation was seen with strawberries ($p=0.404$ for *E.*
385 *multilocularis* and $p= 0.307$ for *E. granulosus s.l.*) and blueberries ($p=1$ for *E. multilocularis*
386 and $p= 0.294$ for *E. granulosus s.l.*). No significant difference ($p=0.776$) was observed between
387 the proportion of *E. granulosus s.s.* DNA contaminating lettuces, strawberries, and blueberries
388 in European endemic areas. However, in European endemic areas, a significantly higher
389 proportion of *E. multilocularis* contamination was found in strawberries and blueberries

390 compared to lettuces ($p=0.0002$). Additionally, a significantly higher proportion of
391 contamination by *E. granulosus s.s.* was observed in strawberries compared to lettuces in
392 Tunisia ($p<0.00001$). A significantly higher proportion of *E. multilocularis* contamination was
393 observed in blueberries compared to lettuces ($p<0.00001$) from Pakistan. Despite having higher
394 proportions of *E. granulosus s.l.* contamination in blueberries than in lettuces in Pakistan,
395 significance level was not reached ($p=0.142$).

396

397 **4. Discussion**

398

399 Due to the long incubation period of *E. multilocularis* and *E. granulosus s.l.*, human CE and
400 AE have been historically considered foodborne diseases, even in the absence of strong
401 evidence of food transmission. Therefore, the acquisition of data on food contamination with
402 *Echinococcus* spp. is crucial to assess the food-borne transmission risk. This study focuses on
403 lettuce and berry, as these products are food items commonly consumed raw, sometimes
404 unwashed, that can easily become contaminated by parasite eggs on the ground and have been
405 considered as a potential source of human infection. In this study, the food items were not
406 washed before being processed in order to provide data on contamination, independently from
407 the washing that should be realized before consumption. To detect the presence of
408 *Echinococcus* spp. DNA in food samples, a sensitive detection method was necessary.
409 Guggisberg et al. (2020) published a one-way sequential sieving method for lettuces, which
410 was followed by a multiplex-PCR designed to distinguish *E. multilocularis*, *E. granulosus s.l.*
411 and others taeniid species (Trachsel et al., 2007). In these two multicenter studies, a real-time
412 PCR detection approach was chosen for *E. multilocularis* and *E. granulosus s.l.* (Knapp et al.,
413 2016; Maksimov et al., 2020). The limit of detection in the conditions laboratory of the
414 multicenter studies was evaluated for *E. multilocularis* in lettuces and also for the first time in

415 berries. Only a minor adjustment to the method was required to remove the eggs without
416 damaging the berries, which could have complicated further filtration steps. In this study, it is
417 assumed that the detected parasite DNA originates from eggs due to the mesh size used to filter
418 the samples. The method demonstrated high sensitivity, detecting up to three eggs in lettuce
419 and berry samples, and even one or two eggs in very high proportions of spiked samples. The
420 sampling of lettuce and berries was designed to minimize the number of samples originating
421 from supermarkets, as any washing or other treatment at manufacturing level would have an
422 impact on the probability to detect parasite DNA. Among the limits of this multicenter study,
423 we should acknowledge possible sampling bias due to: i) the number and varieties of samples
424 collected from one country to another (especially for berries), and ii) geographical origin of
425 samples, generally limited from few areas of the country. Additionally, the percentage of
426 detections gave an overview of the contamination of samples from an area in each country but
427 may not be considered as an absolute value for the country as a whole.

428 The country-based proportions of lettuce and berry samples contaminated by *E. multilocularis*
429 and *E. granulosus s.l.*, observed in this multicenter study were consistent with the known
430 presence of both parasites in these countries. For instance, *E. granulosus s.l.* was not detected
431 in lettuces and berries from countries where the domestic lifecycle of the parasite is not present,
432 such as Denmark, the Netherlands, Germany, and Finland or where the parasite is present at
433 low endemicity such as France (Deplazes et al., 2017). *Echinococcus granulosus s.s.* was
434 predominantly detected in endemic countries where a dog-sheep life cycle occurs, such as Italy,
435 Tunisia and Pakistan. This parasite species was detected in all three endemic Italian regions
436 sampled, confirming a high level of environmental contamination. This is consistent with the
437 strong transmission dynamic between dogs, sheep and humans in the country (Casulli, Abela-
438 Ridder, et al., 2023; Cringoli et al., 2007; Piseddu et al., 2017). In contrast, the parasite was not
439 detected in lettuces from Portugal, where the parasite is present, but at low prevalence in

440 animals and low incidence in humans, respectively (Casulli, Abela-Ridder, et al., 2023; EFSA,
441 2022). However, it should be noted that sampling was only conducted in two local markets
442 from two different villages in Portugal. *Echinococcus granulosus s.s.* was also detected in
443 berries from Latvia and Poland and in one lettuce from Latvia, both eastern European countries
444 where the domestic lifecycle of the parasite is present. In this study, the highest contamination
445 level for *E. granulosus s.s.* was detected from Tunisia, in both lettuces and strawberries. The
446 strawberries were all grown in Cap Bon, located in the northeast of Tunisia, which is the only
447 region in the country with a milder climate suitable for strawberry production. For lettuce,
448 contaminated samples were collected from different regions of the country, including 16
449 samples in Cap Bon, where three (18.8%) samples tested positive to *E. granulosus s.s.* This
450 may indicate a high level of environmental contamination in the country, which is consistent
451 with the high prevalence reported in both humans and animals in Tunisia (M'Rad et al., 2020).
452 The level of contamination in lettuces from Pakistan was similar to that of lettuces from Italy,
453 but blueberries had a higher contamination rate of 12%. These values from Pakistan and Tunisia
454 are both higher than those from Europe. It should be noted that the only *E. canadensis* (G7)
455 specimen has been identified in one lettuce from Pakistan. These results emphasize the need to
456 investigate the environmental contamination and the agricultural practices to produce specific
457 vegetables and berries in different continents to better understand the potential risk associated
458 to foodborne transmission of human CE.

459 *Echinococcus multilocularis* DNA was detected in food items from seven (Denmark Estonia,
460 France, Germany, Latvia, the Netherlands and Switzerland) of the eight European endemic
461 countries (EFSA, 2022) sampled in this study, as well as in Pakistan, where its presence has
462 also been previously reported (Khan et al., 2020; Khan et al., 2021). The parasite is currently
463 considered as absent from Finland, Norway (except for Svalbard Archipelago) and Portugal but
464 also from Tunisia. In Italy, *E. multilocularis* is only present in the northern part of the country.

465 The current northern distribution of *E. multilocularis* in Italy, represented by Liguria region
466 bordering France and the Trentino-Alto Adige region bordering Austria (Casulli et al., 2005;
467 Massolo et al., 2018), does not overlap with the central-southern regions sampled in this study
468 (Abruzzo, Campania and Sardinia). For this reason, the absence of *E. multilocularis* detection
469 in lettuces and strawberries from Italy was not surprising. In endemic countries of Europe, the
470 prevalence of *E. multilocularis* in red foxes, which can be used as an indirect measure of
471 environmental contamination by eggs, varies significantly from one country to another
472 (Oksanen et al., 2016). Therefore, the absence of detection of *E. multilocularis* in both lettuces
473 and berries from Poland may seem surprising in comparison to results from other endemic
474 countries. Indeed, not only because of the high prevalence reported in red foxes (Karamon et
475 al., 2014), but also due to the high percentage of the reported contamination in forest fruits,
476 vegetables and mushrooms collected from both high and low Polish endemic areas from
477 previous studies (Lass et al., 2015b, 2017). In our study, lettuces from Poland were mainly
478 purchased in local markets, while strawberries were collected from kitchen gardens and
479 blueberries from the forest. This sampling was similar to that carried out in the two previous
480 studies from Poland. However, some concerns were raised on the methodology and potential
481 cross-contamination in those two studies which could draw the results into question (Alvarez
482 Rojas et al., 2018; Lass et al., 2016; Robertson et al., 2016; Torgerson, 2016). In contrast to
483 Poland, 4% of lettuce samples (n=50) and 1.8% of strawberry samples (n=56) from Denmark
484 were found to be contaminated by *E. multilocularis*. Interestingly, such percentages has not
485 been found from an area where high local prevalence of *E. multilocularis* in foxes have been
486 previously described (Petersen et al., 2018), reflecting the patchy distribution of *E.*
487 *multilocularis* at a local scale. This highlights the difficulty in drawing definitive conclusions
488 regarding the different levels of food contamination between countries, especially due to the
489 relatively low number of samples collected in each area. To resume, similar values of around

490 1% *E. multilocularis* DNA were obtained in the three European countries (France, Latvia, and
491 Switzerland), where the parasite was identified in lettuces. These findings also match a prior
492 study from Switzerland, whereby a similar proportion of contaminated 1.3% lettuces (n=157)
493 was found using the same washing and sieving method (Guggisberg et al., 2020). Based on this
494 data, it can be estimated from this study that overall 1% of lettuces sampled in endemic areas
495 of European countries are contaminated by *E. multilocularis*.

496 Among the few previous studies on the detection of *E. multilocularis* in berries, there was no
497 detection in blueberry samples from Estonia (n=21) and Finland (n=21) (Malkamäki et al.,
498 2019). *E. multilocularis* DNA was identified in 20% of raspberry samples from plantations in
499 Poland (Lass et al., 2015a). In contrast, our study detected the parasite in berries from all six
500 European endemic countries, with values frequently exceeding 12%, however none were found
501 in the samples from Poland. Due to the limited sample size of berries tested for *E.*
502 *multilocularis*, the absence of detection of the parasite in the 30 berry samples from Poland is
503 perhaps not surprising. Nor was *E. multilocularis* DNA found in blueberries (n=12) and
504 strawberries (n=11) from the highly endemic country of Switzerland, but one of the six
505 additional raspberry samples tested positive. In this study, the highest contamination level by
506 *E. multilocularis* was obtained from blueberries in Pakistan and raises questions about the
507 endemic level in the country and on the level of hygiene during production. Contaminated
508 samples were collected from local markets, vegetable gardens, and from the wild from all the
509 five different cities of production sampled in Pakistan. The incidence of AE in Pakistan is
510 considered to be very low, with only a few human cases reported and a low occurrence in 3 out
511 of 68 (4.4%) red fox faeces examined from the north of the country (Borhani et al., 2021; Khan
512 et al., 2021). Additional epidemiological data on *E. multilocularis* is required from Pakistan for
513 humans, animals, and foodstuffs, such as berries and vegetables, to obtain a more accurate

514 evaluation of the potential risk of foodborne transmission. Furthermore, the potential
515 contribution of dogs to the lifecycle of *E. multilocularis* must be explored in Pakistan.
516 In addition to *Echinococcus* spp., DNA from several other taeniid species were identified,
517 confirming the ability of taeniid eggs to be mechanically transferred on food. In Europe, besides
518 *Echinococcus* spp., the most frequently identified taeniid species (21 out of the 23 taeniid DNA
519 detections) in this study was from the genus *Hydatigera*, resulting from cat defecation. Our
520 finding is consistent with the identification of kitchen gardens as hotspots for both fox and cat
521 defecation in northeastern France (Bastien et al., 2018). Since only a few cats may sporadically
522 excrete high numbers of *E. multilocularis* eggs (Umhang et al., 2022), this species is considered
523 to have a very low biotic potential and therefore does not significantly contribute to overall *E.*
524 *multilocularis* environmental contamination (Hegglin and Deplazes, 2013; Kapel et al., 2006).
525 Additionally, the *E. multilocularis* eggs obtained from cats are generally considered to be non-
526 infectious, which strongly limits the role of cats in foodborne transmission of AE. In a lettuce
527 sample from Norway, it was impossible to differentiate between *T. serialis* and *T. krabbei* based
528 on the partial 12S gene sequence. Both species are present in the country and are associated
529 with intermediate hosts in wildlife (i.e. rabbits and cervids, respectively) and canids (dogs,
530 foxes or wolves) as the definitive hosts. *Taenia hydatigena* was detected in both lettuces and
531 berries in Tunisia and Pakistan. This maybe expected according to the detection in high
532 proportions of *E. granulosus* s.s. in both countries as these species share the same lifecycle
533 between dogs and livestock. The detection of *T. saginata* in Pakistan is associated with
534 environmental contamination by human feces, as humans are the only definitive host. The
535 presence of *T. multiceps* in Sardinia is consistent with previous reports on the presence of this
536 parasite on this island (Scala et al., 2007). This seems to confirms the hypothesis that dogs,
537 which consume raw sheep offal and live in proximity to lettuce and strawberry fields may be a
538 source of *E. granulosus* s.s. eggs contamination. It is worth noting that *Taenia* worms have a

539 much higher egg production capacity than *Echinococcus* worms (Alvarez Rojas et al., 2018).
540 Nevertheless, the rate of DNA detection from *Echinococcus* was found to be globally
541 comparable to that of other taeniid in lettuces and higher in berries, according to this study. In
542 comparison, this was not the case in Guggisberg et al. (2020) study which detected 10 lettuce
543 samples with DNA of others taeniid species versus two with *E. multilocularis* (n=157) and
544 Federer et al. (2015) found only two *E. granulosus* positives mixed vegetables and fruits
545 samples versus 23 positive for others taeniid species among 141 samples. The use of real-time
546 PCR assays for detection of both *E. multilocularis* and *E. granulosus s.l.* in the present study,
547 compared to classical PCR in the other, may support a relatively higher sensitivity of
548 *Echinococcus* species with real-time PCR assays. However, the diversity of *Taenia* species
549 identified was lower than that in the two Swiss studies, despite using the same classical PCR
550 method. Notably, *Taenia crassiceps* and *Taenia polyacantha*, which are typically found in
551 foxes, were not detected in our study.

552 The highest *E. multilocularis* contamination rate of berries compared to lettuces may be due to
553 a difference in the shapes and surface porosity of these vegetables with different egg retention
554 on the different surfaces. Malkamäki et al. (2019) reported a difference in taeniid egg
555 contamination between two types of berries. Since a difference of contamination was not
556 observed for *E. granulosus s.s.* in Europe, it can be supposed that red foxes are more likely to
557 be close to berries than dogs. In Tunisia, the strawberry samples were exclusively obtained from
558 a region which have a milder climate, potentially suggesting a better survival of eggs even if
559 comparable proportions of lettuces contaminated by *E. granulosus s.s.* were obtained from
560 there. The situation in Pakistan is interesting because it is endemic for both CE and AE, with
561 dogs assumed to be strongly involved in the dispersion of eggs of both parasite species. Once
562 again, the difference in contamination between lettuces and blueberries was observed only for
563 *E. multilocularis*. It cannot be assumed that the proportion of parasitic contamination detected

564 in the different food items can be linearly related to the risk of human infection. This is because
565 many factors were not considered, such as the relative quantities of lettuces and berries eaten
566 by consumers or the different exposure of people due to different cultural habits, like
567 prewashing of vegetables and fruits or the proportion of food imported from endemic countries,
568 to obtain a more accurate risk assessment of human foodborne contamination by *Echinococcus*
569 species.

570 The global contamination rate of taeniid eggs in lettuces (5.3%) and berries (13.3%) suggests
571 that the transfer of taeniid eggs from carnivore feces to food is not uncommon. However,
572 although we assume that eggs are the source of the DNA detected in this study, the viability of
573 such eggs is unknown. To obtain direct evidence and an accurate risk assessment of foodborne
574 human infection by *E. multilocularis* and *E. granulosus s.l.*, it is necessary to firstly confirm
575 the viability of *Echinococcus* eggs in food. Currently, in the absence of sensitive in vitro
576 methods, the most reliable viability test requires the experimental infection of small rodents
577 with *Echinococcus* eggs. The subcutaneous method involving mice has been revealed as the
578 most sensitive inoculation method, rather than intraperitoneal or peroral infection (Alvarez
579 Rojas et al., 2018; Federer et al., 2015).

580 The relative contribution of foodborne infection as compared to other sources (e.g. direct
581 contact with dogs, water, or other environmental sources) is likely to differ between continents
582 for both AE and CE (Torgerson et al., 2020). The detection of *E. multilocularis* and *E.*
583 *granulosus s.l.* in lettuces and berries in this study suggests a potential risk of foodborne human
584 infection. The relative contribution of this risk remains to be determined. In the future it would
585 be also important to prioritize studies on novel viability tests for the eggs isolated in food
586 matrices as well as other studies about parasitic DNA detection in food. Moreover, further
587 studies are necessary to combine the detection of *Echinococcus* eggs in food with other
588 environmental matrices from various continents. This will help to assess different

589 epidemiological contexts and social habits, leading to a better understanding of human
590 infections by *Echinococcus* eggs.

591

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604

605 **Conflict of interest:**

606 The authors declare no conflict of interest.

607

608

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Table 2: Number of cases and proportions of detection of *E. multilocularis*, *E. granulosus sensu lato* and other taeniid species DNA in strawberries from the different countries. The 95% confidence intervals are indicated between brackets. *Proportions were calculated considering only those European countries for which areas endemic for *E. multilocularis* have been sampled (Denmark, Estonia, France, Latvia, the Netherlands, Poland and Switzerland) and European countries where a domestic lifecycle is established for *E. granulosus* (Estonia, France, Italy, Latvia, Poland, Portugal).

| Country (regions) | number of strawberries samples | <i>E. multilocularis</i> | | <i>E. granulosus s.l.</i> | | Other taeniid species | |
|-------------------------|--------------------------------|---|--|--|--|---|--|
| | | number of samples tested positive with <i>E. multilocularis</i> | proportions of positive cases (95% CI) | number of samples tested positive with <i>E. granulosus s.l.</i> | proportions of positive cases (95% CI) | number of samples tested positive with others taeniid species | proportions of positive cases (95% CI) with others taeniid species |
| Denmark | 56 | 1 | 1.8% (0-9.6) | 0 | 0% (0-63.8) | 1 <i>Hydatigera</i> sp. | 1.8% (0-9.6) |
| Estonia | 30 | 5 | 16.7% (5.6-34.7) | 0 | 0% (0-52.2) | 0 | 0% (0-52.2) |
| Finland | 10 | 0 | 0% (0-30.8) | 0 | 0% (0-30.8) | 0 | 0% (0-30.8) |
| France | 54 | 0 | 0% (0-6.6) | 0 | 0% (0-6.6) | 0 | 0% (0-6.6) |
| Italy (Sardinia) | 55 | 0 | 0% (0-6.5) | 1 | 1.8% (0-9.7) | 2 <i>Hydatigera</i> sp. | 3.6% (0.4-12.5) |
| Latvia | 30 | 4 | 13.3% (3.8-30.7) | 0 | 0% (0-11.6) | 0 | 0% (0-11.6) |
| Netherlands | 8 | 1 | 12.5% (0.3-52.7) | 0 | 0% (0-36.9) | 0 | 0% (0-36.9) |
| Poland | 13 | 0 | 0% (0-24.7) | 1 | 7.7% (0.2-36.0) | 0 | 0% (0-24.7) |
| Portugal | 17 | 0 | 0% (0-19.5) | 1 | 5.9% (0.1-28.9) | 0 | 0% (0-19.5) |
| Switzerland | 11 | 0 | 0% (0-28.5) | 0 | 0% (0-28.5) | 0 | 0% (0-28.5) |
| SubTotal Europe* | 284 | 11 | 5.4%* (2.7-9.5) | 3 | 1.5%* (0.3-4.3) | 3 <i>Hydatigera</i> sp. | 1.0 (0.2-3.0) |
| Tunisia | 16 | 0 | 0% (0-20.6) | 13 | 81.3% (54.4-96.0) | 1 <i>T. hydatigena</i> | 6.3% (0.2-30.2) |
| Total | 300 | 11 | 3.7% (1.8-6.5) | 16 | 5.3% (3.1-8.5) | 3 <i>Hydatigera</i> sp., 1 <i>T. hydatigena</i> | 1.3% (0.4-3.3) |

Table 3: Number of cases and proportions of detection of *E. multilocularis*, *E. granulosus sensu lato* and other taeniid species DNA in blueberries from the different countries. The 95% confidence intervals are indicated between brackets. The asterisk refers that the percentage value concerns only known endemic countries for *E. multilocularis*. *Proportions were calculated considering only those European countries for which areas endemic for *E. multilocularis* have been sampled (France, Latvia, the Netherlands, Poland and Switzerland) and European countries where a domestic lifecycle is established for *E. granulosus*(France, Latvia, Poland, Portugal).

| Country (regions) | number of blueberries samples | <i>E. multilocularis</i> | | <i>E. granulosus s.l.</i> | | Other taeniid species | |
|-------------------|-------------------------------|---|--|--|--|---|--|
| | | number of samples tested positive with <i>E. multilocularis</i> | proportions of positive cases (95% CI) | number of samples tested positive with <i>E. granulosus s.l.</i> | proportions of positive cases (95% CI) | number of samples tested positive with others taeniid species | proportions of positive cases (95% CI) with others taeniid species |
| Finland | 8 | 0 | 0% (0-36.9) | 0 | 0% (0-36.9) | 0 | 0% (0-36.9) |
| France | 31 | 1 | 3.2% (0.1-16.7) | 0 | 0% (0-11.2) | 0 | 0% (0-11.2) |
| Latvia | 30 | 4 | 13.3% (3.8-30.7) | 1 | 3.3% (0.1-17.2) | 0 | 0% (0-11.6) |
| Netherlands | 6 | 1 | 16.7% (0.4-64.1) | 0 | 0% (0-45.9) | 0 | 0% (0-45.9) |
| Poland | 3 | 0 | 0% (0-70.8) | 0 | 0% (0-70.8) | 0 | 0% (0-70.8) |
| Portugal | 15 | 0 | 0% (0-21.8) | 0 | 0% (0-21.8) | 0 | 0% (0-21.8) |
| Switzerland | 12 | 0 | 0% (0-26.5) | 0 | 0% (0-26.5) | 0 | 0% (0-26.5) |
| SubTotal | | | | | | | |
| Europe* | 105 | 6 | 7.3% (2.7-15.2) | 1 | 1.3% (0.0-6.9) | 0 | 0% (0-3.5) |
| Pakistan | 25 | 14 | 56.0% (34.9-75.6) | 3 | 12.0% (2.5-31.2) | 1 <i>T. hydatigena</i> , 1 <i>Hydatigera</i> sp. | 8.0% (1.0-26.0) |
| Total | 130 | 20 | 15.4% (9.7-22.8) | 4 | 3.1% (0.8-7.7) | 1 <i>T. hydatigena</i> , 1 <i>Hydatigera</i> sp. | 1.5% (0.2-5.4) |

Table 1: Number of tested positive samples and detection proportions of *Echinococcus multilocularis*, *Echinococcus granulosus sensu lato* and others taeniid species DNA in lettuces from the different countries. The 95% confidence intervals are indicated between brackets. *Proportions were calculated considering only those European countries for which areas endemic for *E. multilocularis* have been sampled (Denmark, France, Germany, Latvia, the Netherlands, Poland and Switzerland) and European countries where a domestic lifecycle is established for *E. granulosus* (France, Italy, Latvia, Poland, Portugal).

| Country (regions) | number of lettuces analysed | <i>E. multilocularis</i> | | <i>E. granulosus s.l.</i> | | Other taeniid species | |
|---|-----------------------------|---|--|--|--|---|--|
| | | number of samples tested positive with <i>E. multilocularis</i> | proportions of positive cases (95% CI) | number of samples tested positive with <i>E. granulosus s.l.</i> | proportions of positive cases (95% CI) | number of samples tested positive with others taeniid species | proportions of positive cases (95% CI) with others taeniid species |
| Denmark | 50 | 2 | 4.0% (0.5-13.7) | 0 | 0% (0-7.1) | 0 | 0% (0-7.1) |
| France | 226 | 3 | 1.3% (0.3-3.8) | 0 | 0% (0-1.6) | 6 <i>Hydatigera</i> sp. | 2.7% (1.0-5.7) |
| Germany (Mecklenburg-Western Pomerania) | 9 | 0 | 0% (0-33.6) | 0 | 0% (0-33.6) | 0 | 0% (0-33.6) |
| Germany (Baden-Württemberg region) | 63 | 0 | 0% (0-5.7) | 0 | 0% (0-5.7) | 1 <i>Hydatigera</i> sp. | 1.6% (0-8.5) |
| Italy (Abruzzo region) | 80 | 0 | 0% (0-4.5) | 1 <i>E. granulosus s.s.</i> | 1.3% (0-6.8) | 1 <i>Hydatigera</i> sp. | 1.3% (0-6.8) |
| Italy (Campania region) | 46 | 0 | 0% (0-7.7) | 3 <i>E. granulosus s.s.</i> | 6.5% (1.4-17.9) | 0 | 0% (0-7.7) |
| Italy (Sardinia region) | 106 | 0 | 0% (0-3.4) | 4 <i>E. granulosus s.s.</i> | 3.8% (1.0-9.4) | 3 <i>Hydatigera</i> sp., 1 <i>T. multiceps</i> | 3.8% (1.0-9.4) |
| Latvia | 62 | 1 | 1.6% (0-8.7) | 1 <i>E. granulosus s.s.</i> | 1.6% (0-8.7) | 1 <i>Hydatigera</i> sp. | 1.6% (0-8.7) |
| Netherlands | 6 | 0 | 0% (0-45.9) | 0 | 0% (0-45.9) | 0 | 0% (0-45.9) |
| Norway | 39 | 0 | 0% (0-9.0) | 0 | 0% (0-9.0) | 1 <i>T. krabbei/serialis</i> | 2.6% (0-13.5) |
| Poland | 74 | 0 | 0% (0-4.9) | 0 | 0% (0-4.9) | 0 | 0% (0-4.9) |
| Portugal | 101 | 0 | 0% (0-3.9) | 0 | 0% (0-3.9) | 0 | 0% (0-3.9) |
| Switzerland | 80 | 1 | 1.3% (0-6.8) | 0 | 0% (0-4.5) | 2 <i>Hydatigera</i> sp. | 2.5% (0.3-8.7) |

| | | | | | | | |
|-------------------------|-------|---|-----------------|---|------------------|---|-----------------|
| SubTotal Europe* | 942 | 7 | 1.2%* (0.5-2.5) | 9 <i>E. granulosus</i> s.s. | 1.3%* (0.6-2.4) | 14 <i>Hydatigera</i> sp., 2 <i>Taenia</i> sp. | 1.7% (1-2.7) |
| Pakistan | 100 | 2 | 2% (0.2-7.0) | 3 <i>E. granulosus</i> s.s., 1 <i>E. canadensis</i> | 4.0% (1.1-9.9) | 5 <i>T. saginata</i> , 3 <i>T. hydatigena</i> , 1 <i>Hydatigera</i> sp. | 9% (4.2-16.4) |
| Tunisia | 75 | 0 | 0% (0-4.8) | 9 <i>E. granulosus</i> s.s. | 12.0% (5.6-21.6) | 3 <i>T. hydatigena</i> | 4% (0.8-11.2) |
| Total | 1,117 | 9 | 0.8% (0.4-1.5) | 21 <i>E. granulosus</i> s.s., 1 <i>E. canadensis</i> | 2.0% (1.2-3.0) | 15 <i>Hydatigera</i> sp., 13 <i>Taenia</i> sp. | 2.5% (0.8-11.2) |