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1 **First identification of *Cryptosporidium parvum* zoonotic subtype IIaA15G2R1 in**
2 **diarrheal lambs in France**

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16

17 **Abstract**

18 To date, no information is available about the presence of *Cryptosporidium* spp. in French
19 sheep, nor their potential role as zoonotic reservoirs. A total of 23 fecal samples were
20 collected from **diarrheic** lambs (< 11 days old) from seven randomly selected farms.
21 *Cryptosporidium*-oocysts were detected microscopically with Direct Immunofluorescence
22 Assays (DFA) in 23/23 (100%) of fecal samples. PCR-RFLP of the *18S rRNA* gene was used
23 to determine species in all samples, and only *Cryptosporidium parvum* was identified. Isolates
24 were subtyped by sequencing the 60 kDa glycoprotein (*gp60*) gene. Two zoonotic subtypes
25 within the IIa subtype family were identified, including IIaA15G2R1 (22/23) and
26 IIaA16G3R1 (1/23). This study reports for the first time the identification and genotyping of
27 zoonotic *C. parvum* subtypes from lambs in France. Sheep could thus play an important role
28 as potential reservoirs for this zoonotic protist.

29 **Key words:** *Cryptosporidium*; Lamb; Sheep; Zoonosis; France.

30

31 **Introduction**

32 *Cryptosporidium* is an obligate intracellular protist parasite infecting a wide range of
33 vertebrate hosts—including humans (Bouzig et al., 2013)—and poses a significant threat to
34 public health. Molecular approaches to genetically characterise *Cryptosporidium* spp. has
35 enhanced an improved understanding of cryptosporidiosis epidemiology (Xiao, 2010a).
36 Clinical symptoms of *Cryptosporidium* infection in young ruminants (calves, lambs, and goat
37 kids) include diarrhea, dehydration, delayed growth, and weight loss, often leading to death,
38 thus resulting in considerable economic losses associated with morbidity and mortality (de
39 Graaf et al., 1999). In addition, young ruminants have been considered as a potential source of
40 human cryptosporidiosis infection in several outbreaks (Xiao, 2010a).

41 Currently, more than 30 validated *Cryptosporidium* species have been described (Osman et
42 al., 2017). Besides *C. parvum*, six *Cryptosporidium* species have been identified in sheep
43 feces, including *C. ubiquitum*, *C. xiaoi*, *C. hominis*, *C. andersoni*, *C. fayeri*, and *C. suis*
44 (Paraud and Chartier, 2012). However, it is not yet known which specific *Cryptosporidium*
45 species/subtypes infect sheep in France. Thus far, many studies have characterized
46 *Cryptosporidium* at a molecular level in French calves (Follet et al., 2011; Ngouanesavanh et
47 al., 2006; Razakandrainibe et al., 2018; Rieux et al., 2014, 2013b, 2013c, 2013a) and goat
48 kids (Ngouanesavanh et al., 2006; Paraud et al., 2014; Rieux et al., 2013d). Little is known
49 about the presence of *Cryptosporidium* spp. in sheep, nor the role the animals may play as
50 reservoirs for these parasites. Therefore, the present work aimed to identify *Cryptosporidium*
51 at a molecular level in lambs from two different French departments (Tarn and Haute-
52 Vienne). Furthermore, through genetic characterization, this study led the authors to
53 investigate the potential of lambs as a zoonotic reservoir for human infection.

54

55 **Materials and Methods**

56 Between November 2018 to April 2019, 23 lamb rectal fecal samples were collected from 7
57 **randomly selected** farms across two French departments: Tarn and Haute-Vienne (Figure 1).
58 In order to perform anonymous sampling, farms were arbitrarily numbered from F1 to F7 and
59 collected stool samples were labelled O1 to O23. The farms included in this study all breed
60 mixed ruminants (cattle, sheep, and goats). Sampled lambs were less than 11 days old, and
61 presented with diarrhea. Fecal samples were individually collected from lambs in plastic
62 containers, and conserved at 4°C until analysis within one week.

63

64 **Microscopy screening**

65 All samples were concentrated from 1 g of original fecal matter as previously described
66 (Castro-Hermida et al., 2005), then screened for the presence of *Cryptosporidium* oocysts by
67 direct immunofluorescence assays (DFA) (MeriFluor® *Cryptosporidium/Giardia*, Meridian
68 Bioscience Europe, Milano, Italy) as indicated by the manufacturer, and including previously
69 described modifications (Mammeri et al., 2018).

70

71 **DNA extraction and PCR amplification**

72 Samples with positive DFA underwent genomic DNA extraction using the QIAamp DNA
73 Stool Mini Kit (Qiagen, France), according to manufacturer's instructions. To disrupt oocyst
74 walls, an initial step of ten freeze-thaw cycles was incorporated into the protocol as
75 previously described (Sahraoui et al., 2019).

76 To detect *Cryptosporidium* spp. in DFA-positive samples, nested PCR was used to amplify an
77 840 bp fragment of *18S rRNA* gene as previously described (Xiao et al., 1999).
78 *Cryptosporidium* species were identified by performing restriction fragment length

79 polymorphism (RFLP) analysis with *SspI* and *MboII* endonucleases on *18S rRNA* PCR
80 products (New England BioLabs, France) as previously described (Feng et al., 2007).
81 Comparison of band patterns with those described before (Feng et al., 2007) was used for the
82 identification of the different *Cryptosporidium* species.

83 *Cryptosporidium parvum* samples were subtyped by nested PCR-sequence analysis of the
84 partial 60 kDa glycoprotein locus (*gp60*), and all positive isolates were sequenced as
85 previously described (Alves et al., 2003). Briefly, *gp60*-PCR products were sequenced on
86 both strands using internal primer sets by Genoscreen (France). Consensus sequences were
87 edited using the BioEdit Sequence Alignment Editor software (version 5.0.6) and compared
88 with published GenBank sequences using the freely-available Basic Local Alignment Search
89 Tool (BLAST) from the National Center for Biotechnology Information (NCBI)
90 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). *C. parvum* subtypes were named using the
91 recommended nomenclature system (Sulaiman et al., 2005; Xiao, 2010b). All *gp60* sequences
92 generated in this study from O1 to O23 isolates were deposited into the Genbank database
93 with accession numbers MN037849-MN37871, respectively.

94 In each PCR reaction, both positive and negative control samples were included. The positive
95 control consisted of DNA extracted from 10^6 *C. parvum* Iowa strain oocysts (Waterborne Inc.,
96 New Orleans, Louisiana, USA) while the negative control was purified water.

97 **Results and Discussion**

98 *Cryptosporidium* species may pose a significant threat to public health. They are well-known
99 pathogens infecting both domesticated farm and companion animals. There is considerable
100 genetic diversity within *Cryptosporidium*, as 30 *Cryptosporidium* species with several
101 different subtypes have been described (Cacciò et al., 2005). However, little is known about
102 *Cryptosporidium* occurrence rates in small ruminants in France. For the first time, this study
103 describes the *C. parvum* *gp60* subtypes isolated from lamb feces samples in France.

104 In this study, DFA was used to screen for the presence of *Cryptosporidium* oocysts, prior to
105 genotyping with PCR. *Cryptosporidium* spp. were detected by DFA in 23/23 (100%) of fecal
106 samples from young diarrhoeic lambs (Table 1). Although the number of samples included in
107 this study is small, this study implicates the *Cryptosporidium* species as a neonatal diarrhea
108 agent, however, other intestinal pathogens (*Escherichia coli*, *Salmonella*, *Coccidia*...) that
109 were not investigated here, could also be diarrhea-causing agents in these lambs. DFA-
110 positive samples indicated that lambs excreted from between 2×10^3 to 9×10^7 oocysts per
111 gram of feces (OPG) via direct oocyst detection (Mean = 8×10^6). These results indicate a
112 high level of oocyst excretion, and are similar to a study performed in France which reported
113 oocyst excretion intensity reaching 8×10^6 oocysts per gram of feces in some calves (Rieux et
114 al., 2013b).

115 In this study, PCR-RFLP and sequence analysis of the *18S rRNA* gene confirmed that only the
116 *C. parvum* species was present in the lambs (23/23) (Table 1) (Figure 2). The success of the
117 PCR technique in all samples could be explained by the high excreted parasite load, which
118 may overcome the effects of any naturally-occurring PCR inhibitors in the feces. On the other
119 hand, oocyst concentration may also facilitate *Cryptosporidium* PCR detection by eliminating
120 those naturally-occurring PCR inhibitors (Elwin et al., 2012).

121 As already mentioned, *C. parvum* was the only species identified in this study, similar to
122 previous small ruminant studies in other countries (Drumo et al., 2012; Goma et al., 2007;
123 Maurya et al., 2013; Mueller-Doblies et al., 2008; Quilez et al., 2008; Tzanidakis et al., 2014).
124 However, even though the *C. xiaoi* species is often reported in small ruminants in other
125 countries, it was not identified in lambs in the current study (Paraud and Chartier, 2012).

126 Subtype analysis using the *C. parvum* 60 kDa glycoprotein locus (*gp60*) (Figure 2. C)
127 revealed both human- and zoonotic-specific subtypes (Sulaiman et al., 2005). In this study,

128 the dominant *C. parvum* isolate subtype present in the lambs was IIAA15G2R1 (n = 22/23),
129 while subtype IIAA16G3R1(1/23) was reported at lower rates (Table 1). Our results are
130 consistent with multiple other sheep studies. In fact, it has been reported that the *C. parvum*
131 IIA subtype family is dominant in countries such as the UK, Poland, and New Guinea
132 (Connelly et al., 2013; Kaupke et al., 2017; Koinari et al., 2014). The identified IIA subtypes
133 pose a real risk to public health, as this family is known to include many potentially zoonotic
134 subtypes (Xiao, 2010a). Of note, in other countries (Spain, Romania, and Australia) the IID
135 subtype family dominate (Díaz et al., 2015; Imre et al., 2013; Quilez et al., 2008; Yang et al.,
136 2014).

137 The predominant IIAA15G2R1 subtype has previously been reported as the most prevalent
138 subtype in calves and humans in many countries (Aita et al., 2015; Alves et al., 2006;
139 Danišová et al., 2016; Díaz et al., 2013; Mawly et al., 2015; Soba and Logar, 2008; Wielinga
140 et al., 2008; Xiao, 2010a), including France (Follet et al., 2011; Rieux et al., 2014, 2013c,
141 2013a), thus highlighting the zoonotic potential of lamb reservoirs. It seems that the
142 IIAA15G2R1 *C. parvum* subtype is hypertransmissible, which may explain its predominance
143 (Feng et al., 2018). Future studies are needed to determine whether this subtype is only
144 isolated from mixed-species breeding, or whether subtype predominance is due to one
145 restricted available host.

146 The IIAA16G3R1 *C. parvum* subtype has been identified in many studies of calves from
147 France (Follet et al., 2011; Razakandrainibe et al., 2018; Rieux et al., 2013a), and in
148 ruminants (calves, lambs, and goat kids) from other locations (Spain, Korea, Australia, and
149 Algeria) (Díaz et al., 2015; Lee et al., 2016; Nolan et al., 2009; Sahraoui et al., 2019). In
150 addition, human *Cryptosporidium* infections, including subtype IIAA16G3R1, have been
151 reported in patients from Denmark and Iran (Kiani et al., 2017; Stensvold et al., 2015).

152 Our results suggest that lambs may also be important reservoirs for *C. parvum* zoonotic
153 subtypes in France. Further investigations are required to determine whether this observation
154 holds true in other parts of the country on a larger geographic scale, preferably with larger
155 sample sizes from different French departments, and different farm management practices, to
156 better understand the epidemiology of cryptosporidiosis in lambs.

157 Sequencing of the *gp60* gene could demonstrate the presence of common subtype families in
158 humans as well as animals. This could provide more information about these potentially
159 zoonotic subtype families and their transmission from livestock.

160 **Conclusion**

161 In conclusion, our findings demonstrate that *C. parvum* infection is a common occurrence in
162 lambs. These data strongly suggest that lambs may be important reservoirs of zoonotic *C.*
163 *parvum* subtypes infecting humans in France. This is also the first report of *C. parvum*
164 subtype infections in French lambs, and could serve as baseline data for further investigations
165 to better understand cryptosporidiosis epidemiology and *C. parvum* subtype diversity in
166 France.

167 **Conflict of interest**

168 The authors declare that they have no conflicts of interest.

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175 **References**

176 Aita, J., Ichikawa-Seki, M., Kinami, A., Yaita, S., Kumagai, Y., Nishikawa, Y., Itagaki, T.,
177 2015. Molecular characterization of *Cryptosporidium parvum* detected in Japanese
178 black and Holstein calves in Iwate Prefecture and Tanegashima Island, Kagoshima
179 Prefecture, Japan. *J. Vet. Med. Sci.* 77, 997–999. [https://doi.org/10.1292/jvms.15-](https://doi.org/10.1292/jvms.15-0082)
180 0082

181 Alves, M., Xiao, L., Antunes, F., Matos, O., 2006. Distribution of *Cryptosporidium* subtypes
182 in humans and domestic and wild ruminants in Portugal. *Parasitol. Res.* 99, 287–292.
183 <https://doi.org/10.1007/s00436-006-0164-5>

184 Alves, M., Xiao, L., Sulaiman, I., Lal, A.A., Matos, O., Antunes, F., 2003. Subgenotype
185 Analysis of *Cryptosporidium* Isolates from Humans, Cattle, and Zoo Ruminants in
186 Portugal. *J. Clin. Microbiol.* 41, 2744–2747. [https://doi.org/10.1128/JCM.41.6.2744-](https://doi.org/10.1128/JCM.41.6.2744-2747.2003)
187 2747.2003

188 Bouzid, M., Hunter, P.R., Chalmers, R.M., Tyler, K.M., 2013. *Cryptosporidium*
189 Pathogenicity and Virulence. *Clin. Microbiol. Rev.* 26, 115–134.
190 <https://doi.org/10.1128/CMR.00076-12>

191 Cacciò, S.M., Thompson, R.C.A., McLauchlin, J., Smith, H.V., 2005. Unravelling
192 *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol.* 21, 430–437.
193 <https://doi.org/10.1016/j.pt.2005.06.013>

194 Castro-Hermida, J.A., Pors, I., Poupin, B., Ares-Mazás, E., Chartier, C., 2005. Prevalence of
195 *Giardia duodenalis* and *Cryptosporidium parvum* in goat kids in western France.
196 *Small Rumin. Res.* 56, 259–264. <https://doi.org/10.1016/j.smallrumres.2004.06.007>

- 197 Connelly, L., Craig, B.H., Jones, B., Alexander, C.L., 2013. Genetic diversity of
198 *Cryptosporidium* spp. within a remote population of Soay Sheep on St. Kilda Islands,
199 Scotland. *Appl. Environ. Microbiol.* 79, 2240–2246.
200 <https://doi.org/10.1128/AEM.02823-12>
- 201 Danišová, O., Valenčáková, A., Petrincová, A., 2016. Detection and identification of six
202 *Cryptosporidium* species in livestock in Slovakia by amplification of SSU and GP60
203 genes with the use of PCR analysis. *Ann. Agric. Environ. Med.* 23, 254–258.
204 <https://doi.org/10.5604/12321966.1203886>
- 205 de Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abbassi, H., Peeters, J.E., 1999. A
206 review of the importance of cryptosporidiosis in farm animals. *Int. J. Parasitol.* 29,
207 1269–1287.
- 208 Díaz, P., Quílez, J., Prieto, A., Navarro, E., Pérez-Creo, A., Fernández, G., Panadero, R.,
209 López, C., Díez-Baños, P., Morrondo, P., 2015. *Cryptosporidium* species and subtype
210 analysis in diarrhoeic pre-weaned lambs and goat kids from north-western Spain.
211 *Parasitol. Res.* 114, 4099–4105. <https://doi.org/10.1007/s00436-015-4639-0>
- 212 Díaz, P., Rota, S., Marchesi, B., López, C., Panadero, R., Fernández, G., Díez-Baños, P.,
213 Morrondo, P., Poglayen, G., 2013. *Cryptosporidium* in pet snakes from Italy:
214 Molecular characterization and zoonotic implications. *Vet. Parasitol.* 197, 68–73.
215 <https://doi.org/10.1016/j.vetpar.2013.04.028>
- 216 Drumo, R., Widmer, G., Morrison, L.J., Tait, A., Grelloni, V., D'Avino, N., Pozio, E.,
217 Cacciò, S.M., 2012. Evidence of host-associated populations of *Cryptosporidium*
218 *parvum* in Italy. *Appl. Environ. Microbiol.* 78, 3523–3529.
219 <https://doi.org/10.1128/AEM.07686-11>
- 220 Elwin, K., Robinson, G., Hadfield, S.J., Fairclough, H.V., Iturriza-Gómara, M., Chalmers,
221 R.M., 2012. A comparison of two approaches to extracting *Cryptosporidium* DNA

- 222 from human stools as measured by a real-time PCR assay. *J. Microbiol. Methods* 89,
223 38–40. <https://doi.org/10.1016/j.mimet.2012.02.006>
- 224 Feng, Y., Ortega, Y., He, G., Das, P., Xu, M., Zhang, X., Fayer, R., Gatei, W., Cama, V.,
225 Xiao, L., 2007. Wide geographic distribution of *Cryptosporidium bovis* and the deer-
226 like genotype in bovines. *Vet. Parasitol.* 144, 1–9.
227 <https://doi.org/10.1016/j.vetpar.2006.10.001>
- 228 Feng, Y., Ryan, U.M., Xiao, L., 2018. Genetic diversity and population structure of
229 *Cryptosporidium*. *Trends Parasitol.* 0. <https://doi.org/10.1016/j.pt.2018.07.009>
- 230 Follet, J., Guyot, K., Leruste, H., Follet-Dumoulin, A., Hammouma-Ghelboun, O., Certad, G.,
231 Dei-Cas, E., Halama, P., 2011. *Cryptosporidium* infection in a veal calf cohort in
232 France: molecular characterization of species in a longitudinal study. *Vet. Res.* 42,
233 116. <https://doi.org/10.1186/1297-9716-42-116>
- 234 Goma, F., Geurden, T., Siwila, J., Phiri, I., Gabriël, S., Claerebout, E., Vercruyssen, J., 2007.
235 The prevalence and molecular characterisation of *Cryptosporidium* spp. in small
236 ruminants in Zambia. *Small Rumin. Res.* 72, 77–80.
- 237 Imre, K., Luca, C., Costache, M., Sala, C., Morar, A., Morariu, S., Ilie, M.S., Imre, M.,
238 Dărăbuș, G., 2013. Zoonotic *Cryptosporidium parvum* in Romanian newborn lambs
239 (*Ovis aries*). *Vet. Parasitol.* 191, 119–122.
240 <https://doi.org/10.1016/j.vetpar.2012.08.020>
- 241 Kaupke, A., Michalski, M.M., Rzeżutka, A., 2017. Diversity of *Cryptosporidium* species
242 occurring in sheep and goat breeds reared in Poland. *Parasitol. Res.* 116, 871–879.
243 <https://doi.org/10.1007/s00436-016-5360-3>
- 244 Kiani, H., Haghighi, A., Seyyedtabaei, S.J., Azargashsb, E., Zebardast, N., Taghipour, N.,
245 Rostami, A., Xiao, L., 2017. Prevalence, clinical manifestations and genotyping of

- 246 *Cryptosporidium* spp. in patients with gastrointestinal illnesses in Western Iran. Iran.
247 J. Parasitol. 12, 169–176.
- 248 Koinari, M., Lymbery, A.J., Ryan, U.M., 2014. *Cryptosporidium* species in sheep and goats
249 from Papua New Guinea. Exp. Parasitol. 141, 134–137.
250 <https://doi.org/10.1016/j.exppara.2014.03.021>
- 251 Lee, S.-H., VanBik, D., Kim, H.-Y., Lee, Y.-R., Kim, J.W., Chae, M., Oh, S.-I., Goo, Y.-K.,
252 Kwon, O.-D., Kwak, D., 2016. Multilocus typing of *Cryptosporidium* spp. in young
253 calves with diarrhea in Korea. Vet. Parasitol. 229, 81–89.
254 <https://doi.org/10.1016/j.vetpar.2016.09.019>
- 255 Mammeri, M., Chevillot, A., Thomas, M., Polack, B., Julien, C., Marden, J.-P., Auclair, E.,
256 Vallée, I., Adjou, K.T., 2018. Efficacy of chitosan, a natural polysaccharide, against
257 *Cryptosporidium parvum* *in vitro* and *in vivo* in neonatal mice. Exp. Parasitol. 194, 1–
258 8. <https://doi.org/10.1016/j.exppara.2018.09.003>
- 259 Maurya, P.S., Rakesh, R.L., Pradeep, B., Kumar, S., Kundu, K., Garg, R., Ram, H., Kumar,
260 A., Banerjee, P.S., 2013. Prevalence and risk factors associated with *Cryptosporidium*
261 spp. infection in young domestic livestock in India. Trop. Anim. Health Prod. 45,
262 941–946. <https://doi.org/10.1007/s11250-012-0311-1>
- 263 Mawly, J.A., Grinberg, A., Velathanthiri, N., French, N., 2015. Cross sectional study of
264 prevalence, genetic diversity and zoonotic potential of *Cryptosporidium parvum*
265 cycling in New Zealand dairy farms. Parasit. Vectors 8, 240.
266 <https://doi.org/10.1186/s13071-015-0855-9>
- 267 Mueller-Doblies, D., Giles, M., Elwin, K., Smith, R.P., Clifton-Hadley, F.A., Chalmers,
268 R.M., 2008. Distribution of *Cryptosporidium* species in sheep in the UK. Vet.
269 Parasitol. 154, 214–219. <https://doi.org/10.1016/j.vetpar.2008.03.021>

- 270 Ngouanesavanh, T., Guyot, K., Certad, G., Le Fichoux, Y., Chartier, C., Verdier, R.-I.,
271 Cailliez, J.-C., Camus, D., Dei-Cas, E., Bañuls, A.-L., 2006. *Cryptosporidium*
272 population genetics: evidence of clonality in isolates from France and Haiti. J.
273 Eukaryot. Microbiol. 53 Suppl 1, S33-36. [https://doi.org/10.1111/j.1550-](https://doi.org/10.1111/j.1550-7408.2006.00166.x)
274 [7408.2006.00166.x](https://doi.org/10.1111/j.1550-7408.2006.00166.x)
- 275 Nolan, M.J., Jex, A.R., Mansell, P.D., Browning, G.F., Gasser, R.B., 2009. Genetic
276 characterization of *Cryptosporidium parvum* from calves by mutation scanning and
277 targeted sequencing - zoonotic implications. Electrophoresis 30, 2640–2647.
278 <https://doi.org/10.1002/elps.200900071>
- 279 Osman, M., El Safadi, D., Benamrouz-Vanneste, S., Cian, A., Moriniere, R., Gantois, N.,
280 Delgado-Viscogliosi, P., Guyot, K., Bosc, S., Chabé, M., Petit, T., Viscogliosi, E.,
281 Certad, G., 2017. Prevalence, transmission, and host specificity of *Cryptosporidium*
282 spp. in various animal groups from two French zoos. Parasitol. Res. 116, 3419–3422.
283 <https://doi.org/10.1007/s00436-017-5645-1>
- 284 Paraud, C., Chartier, C., 2012. Cryptosporidiosis in small ruminants. Small Rumin. Res.,
285 Special issue: Specificities of parasitism in goats and sheep: interactions with nutrition
286 and control strategies 103, 93–97. <https://doi.org/10.1016/j.smallrumres.2011.10.023>
- 287 Paraud, C., Pors, I., Rieux, A., Brunet, S., 2014. High excretion of *Cryptosporidium*
288 *ubiquitum* by peri-parturient goats in one flock in western France. Vet. Parasitol. 202,
289 301–304. <https://doi.org/10.1016/j.vetpar.2014.03.024>
- 290 Quilez, J., Torres, E., Chalmers, R.M., Hadfield, S.J., del Cacho, E., Sanchez-Acedo, C.,
291 2008. *Cryptosporidium* Genotypes and Subtypes in Lambs and Goat Kids in Spain.
292 Appl. Environ. Microbiol. 74, 6026–6031. <https://doi.org/10.1128/AEM.00606-08>
- 293 Razakandrainibe, R., Diawara, E.H.I., Costa, D., Goff, L.L., Lemeteil, D., Ballet, J.J.,
294 Gargala, G., Favennec, L., 2018. Common occurrence of *Cryptosporidium hominis* in

- 295 asymptomatic and symptomatic calves in France. PLoS Negl. Trop. Dis. 12,
296 e0006355. <https://doi.org/10.1371/journal.pntd.0006355>
- 297 Rieux, A., Chartier, C., Pors, I., Delafosse, A., Paraud, C., 2013a. Molecular characterization
298 of *Cryptosporidium* isolates from high-excreting young dairy calves in dairy cattle
299 herds in Western France. Parasitol. Res. 112, 3423–3431.
300 <https://doi.org/10.1007/s00436-013-3520-2>
- 301 Rieux, A., Chartier, C., Pors, I., Paraud, C., 2013b. Dynamics of excretion and molecular
302 characterization of *Cryptosporidium* isolates in pre-weaned French beef calves. Vet.
303 Parasitol. 195, 169–172. <https://doi.org/10.1016/j.vetpar.2012.12.043>
- 304 Rieux, A., Paraud, C., Pors, I., Chartier, C., 2014. Molecular characterization of
305 *Cryptosporidium* isolates from beef calves under one month of age over three
306 successive years in one herd in western France. Vet. Parasitol. 202, 171–179.
307 <https://doi.org/10.1016/j.vetpar.2014.03.004>
- 308 Rieux, A., Paraud, C., Pors, I., Chartier, C., 2013c. Molecular characterization of
309 *Cryptosporidium* isolates from pre-weaned calves in western France in relation to age.
310 Vet. Parasitol. 197, 7–12. <https://doi.org/10.1016/j.vetpar.2013.05.001>
- 311 Rieux, A., Paraud, C., Pors, I., Chartier, C., 2013d. Molecular characterization of
312 *Cryptosporidium* spp. in pre-weaned kids in a dairy goat farm in western France. Vet.
313 Parasitol. 192, 268–272. <https://doi.org/10.1016/j.vetpar.2012.11.008>
- 314 Sahraoui, L., Thomas, M., Chevillot, A., Mammeri, M., Polack, B., Vallée, I., Follet, J., Ain-
315 Baaziz, H., Adjou, K.T., 2019. Molecular characterization of zoonotic
316 *Cryptosporidium* spp. and *Giardia duodenalis* pathogens in Algerian sheep. Vet.
317 Parasitol. Reg. Stud. Rep. 16, 100280. <https://doi.org/10.1016/j.vprsr.2019.100280>

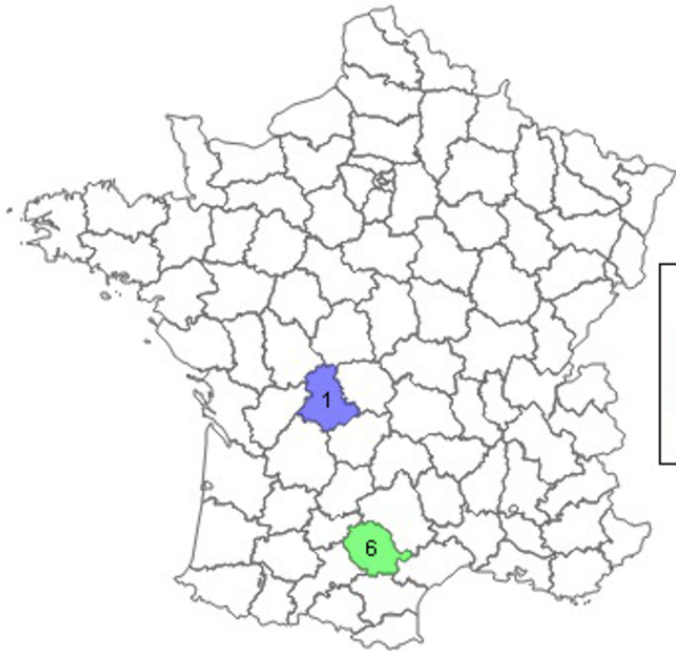
- 318 Soba, B., Logar, J., 2008. Genetic classification of *Cryptosporidium* isolates from humans and
319 calves in Slovenia. *Parasitology* 135, 1263–1270.
320 <https://doi.org/10.1017/S0031182008004800>
- 321 Stensvold, C.R., Ethelberg, S., Hansen, L., Sahar, S., Voldstedlund, M., Kemp, M.,
322 Hartmeyer, G.N., Otte, E., Engsbro, A.L., Nielsen, H.V., Mølbak, K., 2015.
323 *Cryptosporidium* infections in Denmark, 2010-2014. *Dan. Med. J.* 62.
- 324 Sulaiman, I.M., Hira, P.R., Zhou, L., Al-Ali, F.M., Al-Shelahi, F.A., Shweiki, H.M., Iqbal, J.,
325 Khalid, N., Xiao, L., 2005. Unique Endemicity of Cryptosporidiosis in Children in
326 Kuwait. *J. Clin. Microbiol.* 43, 2805–2809. [https://doi.org/10.1128/JCM.43.6.2805-](https://doi.org/10.1128/JCM.43.6.2805-2809.2005)
327 [2809.2005](https://doi.org/10.1128/JCM.43.6.2805-2809.2005)
- 328 Tzanidakis, N., Sotiraki, S., Claerebout, E., Ehsan, A., Voutzourakis, N., Kostopoulou, D.,
329 Stijn, C., Vercruyse, J., Geurden, T., 2014. Occurrence and molecular
330 characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in sheep and goats
331 reared under dairy husbandry systems in Greece. *Parasite Paris Fr.* 21, 45.
332 <https://doi.org/10.1051/parasite/2014048>
- 333 Wielinga, P.R., de Vries, A., van der Goot, T.H., Mank, T., Mars, M.H., Kortbeek, L.M., van
334 der Giessen, J.W.B., 2008. Molecular epidemiology of *Cryptosporidium* in humans
335 and cattle in The Netherlands. *Int. J. Parasitol.* 38, 809–817.
336 <https://doi.org/10.1016/j.ijpara.2007.10.014>
- 337 Xiao, L., 2010a. Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol.* 124,
338 80–89. <https://doi.org/10.1016/j.exppara.2009.03.018>
- 339 Xiao, L., 2010b. Molecular epidemiology of cryptosporidiosis: An update. *Exp. Parasitol.*,
340 *Cryptosporidium* and other waterborne protozoa 124, 80–89.
341 <https://doi.org/10.1016/j.exppara.2009.03.018>

- 342 Xiao, L., Morgan, U.M., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson,
343 R.C.A., Fayer, R., Lal, A.A., 1999. Genetic Diversity within *Cryptosporidium parvum*
344 and Related *Cryptosporidium* Species. *Appl. Environ. Microbiol.* 65, 3386–3391.
- 345 Yang, R., Jacobson, C., Gardner, G., Carmichael, I., Campbell, A.J.D., Ng-Hublin, J., Ryan,
346 U., 2014. Longitudinal prevalence, oocyst shedding and molecular characterisation of
347 *Cryptosporidium* species in sheep across four states in Australia. *Vet. Parasitol.* 200,
348 50–58. <https://doi.org/10.1016/j.vetpar.2013.11.014>
- 349

1 **Figures**

2 **Figure 1.** Geographical map of pre-weaned lamb faecal sampling locations in French
3 departments.

4 The number of sampled farm from each French department (administrative department
5 number-department name) was: 81-Tarn: n=6, and 87-Haute-Vienne: n= 1. The map was
6 edited using *Cartes et Données*- ® Articque ([https://www.articque.com/solutions/cartes-et-](https://www.articque.com/solutions/cartes-et-donnees/)
7 [donnees/](https://www.articque.com/solutions/cartes-et-donnees/)).



French department



81-Tarn



87-Haute-Vienne



Table 1. Molecular characterization of *Cryptosporidium* from clinically-affected pre-weaned French lamb, including age and parasitic burden (oocysts shedding) data.

Sampled lambs and farms included in this study were arbitrary designed from O1 to O23, and from F1 to F7, respectively. Department number: 81: Tarn, and 87: Haute-Vienne; n = total number of samples from each farm; OPG: oocysts per gram of feces.

No. of samples (n = 23)	Department number	Farm (n)	Age (Days)	OPG	<i>C. parvum</i> subtype	Accession numbers	
O1	81 (Tarn)	F1 (1)	10	2 x 10 ³	IIaA15G2R1	MN37849	
O2		F2 (1)	10	8 x 10 ⁴		MN37850	
O12		F4 (1)	7	2 x 10 ⁷		MN37860	
O13			8	1 x 10 ⁶		MN37861	
O14			8	8 x 10 ⁶		MN37862	
O15		F5 (5)	8	3 x 10 ⁶		MN37863	
O16			8	9 x 10 ⁶		MN37864	
O17			8	7 x 10 ⁵		MN37865	
O18		F6 (1)	5	2 x 10 ⁶		IIaA15G2R1	MN37866
O19			5	9 x 10 ⁷		MN37867	
O20			5	3 x 10 ⁶		MN37868	
O21		F7 (5)	10	7 x 10 ⁶		IIaA15G2R1	MN37869
O22			8	3 x 10 ⁶		MN37870	
O23			6	1 x 10 ⁷		MN37871	
O3	87 (Haute-Vienne)		10	7 x 10 ⁵	IIaA15G2R1	MN37851	
O4			5	1 x 10 ⁶		MN37852	
O5			6	1 x 10 ⁶		MN37853	
O6			4	5 x 10 ⁵		MN37854	
O7		F3 (9)	6	1 x 10 ⁵		MN37855	
O8			2	1 x 10 ⁵		MN37856	
O9			2	2 x 10 ⁵		MN37857	
O10			5	5 x 10 ⁴		MN37858	
O11			6	8 x 10 ⁴		MN37859	