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1 **Effect of temperature on the development of the free-living stages of horse cyathostomins**

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27 **Abstract**

28 Cyathostomins are considered as the most prevalent and pathogenic parasites of grazing horses. The
29 development on pastures of the free-living stages of these gastrointestinal worms is particularly
30 influenced by outdoor temperature. Understanding the bionomics of free-living stages is an
31 important prerequisite to implement mathematical models designed to assess the parasitic risk for
32 grazing equids. The aim of this study was to assess the effect of 3 constant temperatures under
33 laboratory conditions ($10\pm 1^{\circ}\text{C}$, $23\pm 2^{\circ}\text{C}$, $30\pm 2^{\circ}\text{C}$) and one fluctuating temperature under outdoor
34 conditions (mean: $17\pm 4^{\circ}\text{C}$) on the minimum time taken by cyathostomin eggs to develop into
35 first/second stage larvae (L1/L2) then into infective third stage larvae (L3) in horse faeces.

36 According to the temperatures, the minimum time taken by eggs to develop into L1/L2 was between
37 1 and 3 days and into L3 between 4 and 22 days. At 10°C , the development time of eggs into L3 was
38 the longest and at 30°C the fastest. The results were consistent with historically available data and
39 their compilation should lead to the improvement of parameterised models assessing the parasitic
40 risk period in grazing equids.

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42 **Keywords:** Horse, cyathostomin, free-living stages, larval development, temperature

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53 **Introduction**

54 Cyathostomins (Small Strongyles: Nematoda, Strongylida) are considered as the most common
55 parasites in grazing equids and usually the most prevalent cause of disease, ill-thrift and poor
56 performance (Collobert-Laugier et al., 2002; Corning, 2009). The cyathostomin group consists of
57 around 50 species, but most infections comprise only 5–10 common species (Lyons et al., 1999).
58 Heavy infections can cause clinical strongylosis, especially in young animals (up to 2-3 years of age),
59 the spring larval cyathostominosis (due to the simultaneous mass emergence of encysted fourth-
60 stage larvae from the intestinal mucosa) being the most clinically severe leading sometimes to death.
61 Such clinical infections occur in heavily infected animals which have been exposed to high pasture
62 larval levels (Corning, 2009). The severity of these infections is therefore related to the factors having
63 an effect on the development and survival of the free living stages on pasture, temperature being
64 considered as the most important natural factor affecting those pre-parasitic stages, followed by
65 moisture and sunlight (ultraviolet light) (Nielsen et al., 2007; Van Dijk et al., 2009). The studies
66 focusing on the effect of temperature on the development of cyathostomins were carried out several
67 decades ago and for some of them with artificial culture media (e.g., De Blicke et al., 1923;
68 Hummelinck et al., 1946; Ogbourne, 1970; Rupasinghe, 1975; Mfitilodze and Hutchinson, 1987).
69 Furthermore, since the introduction of highly efficacious anthelmintic drugs, the biology of free-living
70 stages of equines strongyles has received scant attention and more interest should be paid to the
71 actual larval biology in relation to climatic factors (Nielsen et al., 2007).

72 This relationship between larval biology and climatic factors is essential to develop model that mimic
73 the parasite life cycle and the accumulation of free-living stages on pastures, such model allowing to
74 better understand and predict the epidemiology of these infections. A model was carried out to
75 describe the dynamics on pastures of the free-living stages of equine cyathostomins using the aged
76 data (Leathwick et al., 2015).

77 The aim of this study was therefore to assess the effect of temperature (constant at the laboratory or
78 fluctuating under outdoor conditions) on the minimum time taken by cyathostomins eggs to develop

79 into first/second stage larvae (L1/L2) then into infective third stage larvae (L3) in horse faeces. These
80 results were compared to the available data to determine whether it could be necessary to update
81 the parameterization of existing models and/or to populate the data for the construction of new
82 models.

83

84 **Materials and methods**

85 Faeces of two 10 years old mares (trotters) were collected in September 2020. These mares were
86 naturally infected with cyathostomins and had been grazing since March at the station of the French
87 horse and riding institute (www.ifce.fr) in Normandy. From individual faeces samples, cyathostomin
88 egg count was carried out through the modified McMaster technique with 15 eggs per gram
89 sensitivity (Raynaud, 1970). Both animals presented a number of eggs of cyathostomins per gram of
90 faeces > 500 (705 and 1545, respectively) classifying them as high egg shedders according to the
91 guidelines suggested by the American Association of Equine Practitioners (AAEP Parasite Control
92 Guidelines, 2019). Subsequently, individual faecal samples were pooled and 80 Petri dishes
93 containing each 5 g of faeces were prepared and divided into four batches of 20 Petri dishes.

94 To explore a wide temperature range, three batches were placed in three different incubators set at
95 $10\pm 1^{\circ}\text{C}$, $23\pm 2^{\circ}\text{C}$ and $30\pm 2^{\circ}\text{C}$. These temperatures corresponded to the ovens available in our
96 laboratory. Moreover, 10°C and 23°C are respectively close to the average temperatures in France at
97 the beginning of the grazing season (March-April) and in the middle of the season (July-August) while
98 daytime temperatures often reach 30°C (<https://meteofrance.fr>). The last batch was placed outside
99 in a shelter protected from the rain and in indirect sunlight (starting date: 15/09/2020, Fig. S1). The
100 temperature at the location of the samples was taken and recorded every 30 minutes.

101 In each Petri dish, a constant faecal humidity level was maintained using a pH 7 commercial mineral
102 water (Volvic®) as previously described (Collobert-Laugier et al., 1999). Faeces were then slightly re-
103 moistened each day with a few drops of water. In addition, jars of mineral water were deposited in
104 the incubators next to the petri dishes.

105 Every day from days 1 to 16, then on days 20, 22, 24 and 28, one of the 20 Petri dishes was collected
106 from each batch and faeces were sedimented for 24 hours at room temperature in Baermann
107 apparatus (Baermann, 1917) to collect potential larvae.

108 Microscopical examination was carried out to detect larvae of cyathostomins and differentiate L1/L2
109 from L3 (Russel, 1948; Belivaqua et al., 1993). To facilitate larvae identification under the
110 microscope, 10 μ L of Lugol's iodine solution was added to 100 μ L of the resulting filtrate to
111 immobilize larvae.

112

113 **Results**

114 In coproscopical analysis and larval cultures, only eggs and larvae of *Cyathostomum* spp. were
115 identified.

116 According to the temperatures, the minimum time taken by cyathostomin eggs to develop into L1/L2
117 was between 1 and 3 days and into L3 between 4 and 22 days (Table 1). At 10°C, the development
118 time of eggs into L3 was the longest and at 30°C the fastest. With the outdoor conditions
119 (temperatures ranging from 11-25°C with a mean of 17°C, Fig.S1), the results obtained were similar
120 to those with 23°C.

121 Figure 1 summarises the data published in various countries worldwide on the minimum time taken
122 by strongyle eggs in faeces to develop into L3 at different temperatures. These data were published
123 between 1923 and 1987, and have been supplemented by our own results. Infective larvae were
124 recovered from faeces incubated at temperatures between 7.5 and 37°C. Globally, larval maturing
125 time was very slow at the lowest temperature and fast at the highest temperature (33-48 days at 7.5-
126 8°C *versus* 1.7 days at 37°C, Fig. 1). However, important differences between published data in terms
127 of minimum time taken by eggs in faeces to develop into L3 were observed at low temperatures: 15
128 days at 8°C (min: 33 days, max: 48 days) and 10 days at 10 °C (min: 15 days, max: 25 days),

129 respectively (Figure 1). Besides, at high temperatures (around 26-27°C), a variation ranging from 1 to
130 8.5 days was also identified.

131

132 **Discussion**

133 In this study, we examined the effect of temperature on the development of cyathostomin eggs into
134 L3 in order to estimate the time of onset of infective stages, with three different constant
135 temperatures and fluctuating temperatures under outdoor condition. In our protocol, a long
136 incubation time was allowed, especially for the low temperature (10°C), in order to avoid mistakenly
137 concluding that larval development had been arrested. Indeed, it had already been observed
138 previously that the development of eggs into L3 could take 48 days at 8°C (Shagalin, 1960).

139 In our results, the long delay between the appearance of L1/L2 and L3 stages at 10°C suggests that
140 the slowing in larval maturing was linked to a blocking development during the L1/L2 stages rather
141 than a longer hatching time.

142 When considering together data from several previous studies culturing eggs in which their
143 development into L3 took place in faeces, in overall, no difference in development time was
144 observed according to i) the year of the study, ii) the climate of the country in which parasites were
145 collected (continental/oceanic/tropical/Mediterranean/temperate climates) and iii) the strongyle
146 species (*Cyathostomum* spp. versus other strongyle species + unidentified).

147 However, data from the literature would indicate that differences between species are possible. As
148 an example, *Strongylus vulgaris* (large strongyle) was found to require a much longer time to develop
149 in faeces from L2 to L3 and then to complete its development than the other strongyle species
150 (Ogbourne, 1972). In the Figure 1, at around 27°C, *Strongylus vulgaris* was also the slowest to
151 develop from eggs into L3 (8.5 days; Baruš, 1958) in comparison to *Trichonstrongylus axei* (4 days;
152 Mirzayans, 1969) and *Triodontophorus tenuicollis* (4 days; Ortlepp, 1925).

153 In an artificial culture medium, Rupasinghe (1975) also observed that *Cylicostomum nassatus* differed
154 from other *Cyathostomum* spp. and was the quickest strongyle to become infective.

155 In our study, due to the absence of identification at the species level within *Cyathostomum* spp., it
156 was not possible to compare the development between individual species in faeces. In addition, to
157 establish the comparison of our results with the available published data, we did not include the data
158 obtained from experimental studies using artificial culture medium (e.g., faecal agar, water) because
159 the time of development of strongyle eggs/larvae has been showed to be slower on artificial culture
160 medium than in faeces (Hummelinck, 1946; Ogbourne, 1970). Besides, in artificial medium, the
161 lowest temperature at which the development into L3 took place was higher than in faeces (12°C
162 *versus* 7.5°C respectively), probably due to an inadequate food supply (e.g., the bacteria added to the
163 medium multiply too slowly) (Ogbourne, 1970; Rupasinghe, 1975).

164 Despite a strong relationship between temperature and development time, some variabilities
165 between data might also be explained by differences in experimental protocols (e.g. eggs directly
166 extracted from adult parasites *versus* from faeces, that is to say after a certain period of time after
167 the laying of the female parasites).

168 In conclusion, despite the lack of replica our results are consistent with previously published findings
169 and supplement current knowledge on the bionomics of cyathostomins. The use of two high egg
170 shedders to collect eggs may have facilitated the observation of larvae in the filtrate/sediment from
171 the Baermann apparatus. An important point revealed by this study is that the different species of
172 strongyle/cyathostomin encountered throughout the world seem to share the same global
173 relationship between incubation temperature and the time required for development into L3.

174 The compilation of these data should help to improve the parameterisation of models assessing the
175 infective larval pressure on paddocks and then the parasitic risk period for grazing equids in order to
176 help owners to optimise their use of anthelmintics.

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181 **Ethical statement:** The procedures involving horses received approval from the Ethics Committee of
182 Normandy (France), DGRI agreement APAFIS#2020041615267486. Animal studies were compliant
183 with all the applicable provisions established by European directive 2010/63/UE. All the methods
184 were performed by approved staff members in accordance with the relevant standard operating
185 procedures approved by the abovementioned ethics committees. All the animals used in this study
186 were handled in strict accordance with good clinical practices and all efforts were made to reduce
187 animal stress.

188

189 **Declaration of competing interest:** The authors declare no potential conflicts of interest with respect
190 to the research, authorship, publication of this article and/or financial and personal relationships that
191 could inappropriately influence this work.

192

193 **Data Availability Statement:** The data that support the findings of this study are available from the
194 corresponding author upon reasonable request.

195

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199

200 **Authors' contributions:** AM conducted and supervised this study. All authors participated in the
201 protocol development. MD and LB collected the faeces. CS and MB carried out the laboratory
202 analysis. AM, LH and CS performed the data analysis. AM and LH drafted the article. All the authors
203 critically revised the article and approved the final version before submission.

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209

210 **References**

211

212 American Association of Equine Practitioners (AAEP), 2019. Parasite Control Guidelines.

213 <http://www.aaep.org/info/parasite-control-guidelines-231>

214

215 Baermann, G., 1917. Eine einfache Methode zur Auffindung von Ankylostomum – (Nematoden) –

216 Larven in Erdproben (in German). Weltevreden Batavia, Geneesk. Lab. Feestbundel. 41-47.

217

218 Baruš, V., 1958. Vývojové cykly některých cizopasných hlístic našich koní (in Czech). Sb. Vysoké šk.

219 zeměd. lesn. Brně, 1, 41-53.

220

221 Belivaqua, C.M.L., Rodrigues, M. de L., Concordet, D., 1998. Identification of infective larvae of some

222 common nematode strongylids of horses. Revue Méd. Vét. 144(12), 989-995.

223

224 Collobert-Laugier, C., Bernard-Brisseau, N., Lamidey, C., Hubert, J., Kerboeuf, D., Clément, F.,

225 Flochlay, A., Blond-Riou, F., 1999. Identification of benzimidazole-resistant cyathostomes of horses in

226 Normandy (France) and efficacy of moxidectin against these resistant populations. Proceedings of

227 the 26th world veterinary congress, 23-26th September, Lyon, France.

228

229 Collobert-Laugier, C., Hoste, H., Sevin, C., Dorchies, P., 2002. Prevalence, abundance and site

230 distribution of equine small strongyles in Normandy, France. Vet. Parasitol. 110, 77-83.

231 [https://doi.org/ 10.1016/s0304-4017\(02\)00328-x](https://doi.org/10.1016/s0304-4017(02)00328-x)

232

233 Corning, S., 2009. Equine cyathostomins: a review of biology, clinical significance and Therapy. Parasit
234 Vectors. 2:S1. <https://doi.org/10.1186/1756-3305-2-S2-S1>
235

236 De Blicq, L., 1923. Infectie en prophylaxis bij strongylosis van het paard (in Dutch). Proceedings of
237 Nederlandsch Natuur-en Geneeskundig Congres. 19, 188-193.
238

239 Hummelinck, P.W., 1946. Onderzoekingen over de ontwikkelingssnelheid van eieren en larven van
240 paardenstrongyliden (in Dutch). Tijdschr. Diergeneeskd. 71, 842-852.
241

242 Leathwick, D.M., Donecker, J.M., Nielsen, M.K., 2015. A model for the dynamics of the free-living
243 stages of equine cyathostomins. Vet. Parasitol. 209(3-4), 210-220.
244 <https://doi.org/10.1016/j.vetpar.2015.02.031>
245

246 Lyons, E.T., Tolliver, S.C., Drudge, J.H., 1999. Historical perspective of cyathostomes: prevalence,
247 treatment and control programs. Vet Parasitol. 85, 97-112. [https://doi.org/10.1016/S0304-](https://doi.org/10.1016/S0304-4017(99)00091-6)
248 [4017\(99\)00091-6](https://doi.org/10.1016/S0304-4017(99)00091-6)
249

250 Mfitalodze, M.W., Hutchinson, G.W., 1987. Development and survival of free-living stages of equine
251 strongyles under laboratory conditions. Vet Parasitol. 23(1-2), 121-133.
252 [https://doi.org/10.1016/0304-4017\(87\)90030-6](https://doi.org/10.1016/0304-4017(87)90030-6)
253

254 Mikačić, D., 1953. Preparasitiski razvoj pripadnika *Strongylidae* iz crijeva konja (in Slovenian).
255 Veterinarski Arhiv. 23, 87-92.
256

257 Mirzayans, A., 1969. The effect of temperature on the development of the eggs and larvae of
258 *Trichostrongylus Axei*. Br. Vet. J. 125(12). [https://doi.org/10.1016/s0007-1935\(17\)48620-9](https://doi.org/10.1016/s0007-1935(17)48620-9)

259

260 Nielsen, M.K., Kaplan, R.M., Thamsborg, S.M., Monrad, J., Olsen, S.N., 2007. Climatic influences on
261 development and survival of free-living stages of equine strongyles: Implications for worm control
262 strategies and managing anthelmintic resistance. *Vet. J.* 174(1), 23-32.
263 <https://doi.org/10.1016/j.tvjl.2006.05.009>

264

265 Ogbourne, C.P., 1970. Studies on the biology of strongylid worms of the horse. Thesis, University of
266 Bristol.

267

268 Ortlepp, R.J., 1925. Observations on the Life History of *Triodontophorus tenuicollis*, a Nematode
269 Parasite of the Horse. *J. Helminthol.* 3(1), 1-14.

270

271 Raynaud, J.P., 1970. Étude de l'efficacité d'une technique de coproscopie quantitative pour le
272 diagnostic et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Ann.*
273 *Parasitol. (Paris).* 45, 321-342.

274

275 Rupasinghe, D., 1975. Development, Physiological and morphological observations on the free-living
276 and parasitic stages of some strongylid nematodes of the horse. Thesis, University of London.

277

278 Russel, A.F., 1948. The development of helminthiasis in thoroughbred foals. *J. Comp. Pathol. Ther.*
279 58(2), 107-127. [https://doi.org/10.1016/s0368-1742\(48\)80009-3](https://doi.org/10.1016/s0368-1742(48)80009-3)

280

281 Shagalin, S.F., 1960. Razviti lichinok patogennykh nematody loshadi vo vneshnei srede v usloviyakh
282 Turkmenistana (in Russian). *Trudy Inst. Zool. i Parazitol., Akad. Nauk. Turkmen. SSR.* 5, 237-242.

283

284 Van Dijk, J., de Louw, M.D.E., Kalis, L.P.A., Morgan, E.R., 2009. Ultraviolet light increases mortality of
285 nematode larvae and can explain patterns of larval availability at pastures. *Int. J. Parasitol.* 39(10),
286 1151-1156. <https://doi.org/10.1016/j.ijpara.2009.03.004>

287

288 Velichkin, P.A., 1954. Intravital diagnosis of delafondiasis, alfortiasis, strongylasis and the
289 trichonematiniases of horses from their invasive larvae (in Russian). *IZV Mosk. Zootek. Inst. Konev.* 9,
290 22-28.

291

292 Wagner, G.G., 1938. *Veter. Med. Nachr. Sonderh. Kongr. Zurich.* (Cited by Hummelinck, P., 1946.
293 Investigation of the eggs of horse strongyles. *Tijdschr. Diergeneeskd.* 71, 411-427).

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310 **Table 1.** Minimum time taken by cyathostomin eggs to develop into first/second stage larvae (L1/L2)
311 and then into infective third stage larvae (L3) at different temperatures

Temperature (°C)	L1/L2	Infective larvae (L3)
10	3 days	22 days
23	1 day	5 days
30	2 days	4 days
Outdoor condition ^a	1 day	5 days

312

313 ^aMean: 17±4°C

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325 **Figure captions**

326 **Figure 1.** Minimum time taken by strongyles eggs in faeces to develop into infective third stage
327 larvae (L3) depending on incubation temperature. Data collected from the literature and
328 supplemented by our own results.

329

330 ¹ Ortlepp, 1925; Ogbourne, 1970

331 ² Mfitalodze and Hutchinson, 1987

332 ³ Mikačić, 1953

333 ⁴ Our results

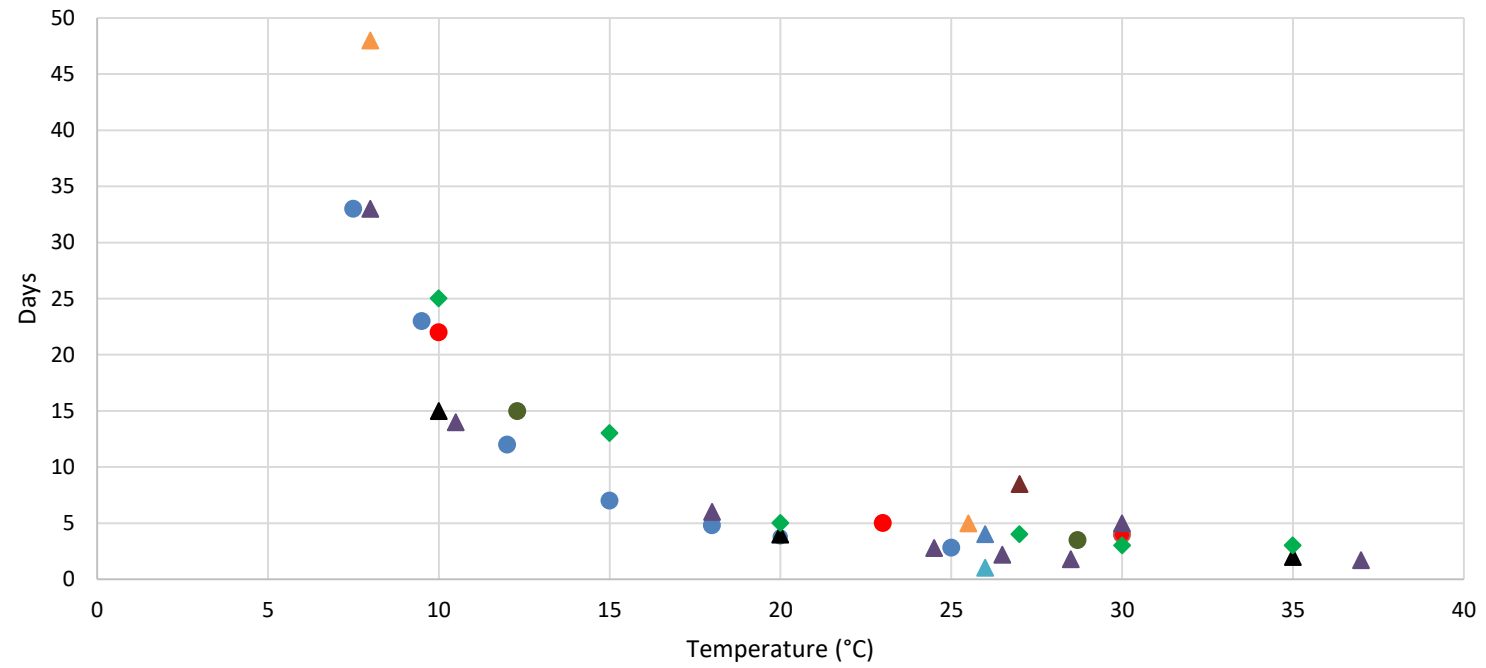
334 ⁵ De Blicck, 1923; Hummelinck, 1946

335 ⁶ Velichkin, 1954; Shagalin, 1960

336 ⁷ Wagner, 1938

337 ⁸ Baruš, 1958

338 ⁹ Mirzayans, 1969



■ United Kingdom¹
 ■ Australia²
 ■ Croatia³
 ■ France⁴
 ■ Netherlands⁵
 ■ Russia⁶
 ■ Switzerland⁷
 ■ Czech republic⁸
 ■ Iran⁹
○ *Cyathostomum* spp.
 △ Strongylidae family (unidentified sp., *Strongylus* spp., *Triodontophorus* spp.)
◇ *Trichostrongylus* sp.