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Implications of between-isolate variation for climate change impact modelling of *Haemonchus contortus* populations

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1 **Implications of phenotypic trait variation for climate change impact modelling of**

2 ***Haemonchus contortus* populations**

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20 **Abstract**

21 The impact of climate change on parasites and parasitic diseases is a growing concern and numerous
22 empirical and mechanistic models have been developed to predict climate-driven spatial and
23 temporal changes in the distribution of parasites and disease risk. Geographical variation in parasite
24 phenotype could undermine the application of such models at broad spatial scales. Seasonal
25 variation in the transmission of the haematophagous gastrointestinal nematode *Haemonchus*
26 *contortus*, one of the most pathogenic helminth species infecting sheep and goats worldwide, is
27 primarily determined by the impact of environmental conditions on the free-living stages. To
28 evaluate variability in the development success and mortality of the free-living stages of *H. contortus*
29 and the impact of this variability on future climate impact modelling, three isolates of diverse
30 geographical origin were cultured at a range of temperatures between 15°C and 37°C to determine
31 their development success compared with simulations using the GLOWORM-FL *H. contortus* model.
32 No significant difference was observed in the developmental success of the three isolates of *H.*
33 *contortus* tested, nor between isolates and model simulations. However, development success of all
34 isolates at 37°C was lower than predicted by the model, suggesting the potential for overestimation
35 of transmission risk at higher temperatures, such as those predicted under some scenarios of climate
36 change. Recommendations are made for future climate impact modelling of gastrointestinal
37 nematodes.

38

39

40 1. Introduction

41 The impact of climate change on the distribution of parasites and parasitic diseases is a growing
42 concern and empirical and mechanistic models have been developed to predict climate-driven
43 spatial and temporal changes in the distribution of parasites and disease risk (Wall and Ellse, 2011;
44 Rose et al., accepted; Caminade et al., 2015). These models are often employed to make predictions
45 on broad spatial and temporal scales, under the assumption that the underlying determinants of
46 parasite ecology are conserved in time and space.

47 Gastrointestinal nematodes (GINs) infecting ruminants affect host productivity and welfare
48 worldwide (Nieuwhof and Bishop 2005; Charlier et al., 2014) and numerous models aiming to
49 optimise control strategies have been developed (reviewed by Cornell, 2005). In recent years,
50 attention has shifted to the impacts of climate change on GINs and models have been developed
51 that are largely parameterised using data on the average response of parasites to environmental
52 stochasticity (Molnár et al., 2013; Rose et al., 2015). However GINs are genetically diverse within
53 species (Troell et al., 2006a; Hunt et al., 2008; Redman et al., 2008). Phenotypic diversity is also
54 observed but varies by trait (LeJambre and Whitlock, 1976; Troell et al., 2006b; Hunt et al., 2008;
55 Angulo-Cubillán et al., 2010; van Dijk and Morgan, 2010). Regional differences in parasite phenology
56 arising from this variation may undermine modelling efforts if the level of variation is sufficient to
57 result in biologically meaningful disparities between model predictions and parasite behaviour.

58 Seasonal variation in the transmission of the haematophagous GIN *Haemonchus contortus*, one of
59 the most pathogenic GIN species infecting sheep and goats worldwide, is primarily determined by
60 the impact of environmental conditions on the free-living stages. The objectives of this study were
61 therefore to evaluate variability in the development success and mortality of the free-living stages of
62 *H. contortus* isolates at a range of temperatures and assess the impact of this variability on the
63 output of a model developed to simulate the climate-dependent population dynamics of *H.*
64 *contortus*.

65 **2. Methods**

66 *2.1 H. contortus isolates*

67 Three pure isolates of *H. contortus* were used; MHco3(ISE) provided by the Moredun Research
68 Institute, HC1CH provided by the University of Zurich, and the McMaster isolate provided by the
69 Freie Universität Berlin. All isolates were susceptible to anthelmintics. MHco3(ISE) was derived from
70 the ISE *H. contortus* isolate, which itself is derived from the SE isolate, thought to have originated
71 from East Africa (Kenya) in the 1950s (Redman et al., 2008; Sargison, 2008). The McMaster isolate
72 has a similar history of laboratory maintenance, having been isolated from sheep in Australia in 1931
73 (Hunt et al., 2008). The HC1CH isolate was purified from naturally infected sheep in the Swiss
74 midland region for *in vitro* anthelmintic resistance tests, and has been maintained under laboratory
75 conditions at the University of Zurich since 2002.

76 *2.2 Collection and transport of faeces containing H. contortus eggs*

77 Faeces containing eggs were collected from donor lambs (infected for reasons other than the
78 present study) using a harness over a 4-24 hour period. Faeces containing eggs of the HC1CH isolate
79 were vacuum packed for preservation (Rinaldi et al., 2015), transported to the University of Bristol
80 by passenger airline and used within 12 hours of arrival (total transit time <48 hours). Faeces
81 containing eggs of the MHco3(ISE) isolate were also vacuum packed, posted by Royal Mail to the
82 University of Bristol and used immediately upon arrival (total transit time <24 hours). Experiments
83 using the McMaster isolate were conducted at Freie Universität Berlin and therefore faeces were
84 used within 8 hours of collection.

85 *2.3 Experiment design*

86 Faeces were mixed thoroughly and a minimum of 3 egg counts conducted using the modified
87 McMaster's method, sensitive to 50 eggs per gram (epg; MAFF, 1986). At the same time, the
88 developmental stage of HC1CH and MHco3(ISE) eggs was recorded as unembryonated (up to late

89 gastrula stage) or embryonated (“comma” stage onwards) to ensure only undeveloped eggs were
90 used.

91 Subsamples of the homogenised faeces weighing 3g each were placed in 6cm diameter petri dishes
92 and incubated at 15°C, 25°C or 37°C for 23, 12 or 5 days respectively. The temperatures were chosen
93 to capture peak of *H. contortus* L3 recovery at 20-25°C and to span the range of maximum summer
94 temperatures experienced throughout the majority of Europe under current conditions (Klein Tank
95 et al., 2002). The upper temperature of 37°C was included to capture extreme high temperatures
96 that may be experienced in the dung due to solar radiation (Hertzberg, H., unpublished data) and
97 predicted future increases in climate extremes (Kovats et al., 2014). Incubation times were derived
98 from time to peak L3 recovery at each temperature, estimated using the GLOWORM-FL model of the
99 population dynamics of *H. contortus* (Figure 1; Rose et al., 2015). A minimum of 5 replicates per
100 temperature, per isolate, were used.

101 Cultures were kept moist throughout experiments by the addition of tap water when condensation
102 no longer formed on the petri dish lid or if faeces appeared to be drying. One MHco3(ISE) replicate
103 was lost at 15°C due to overgrowth of fungal hyphae. L3 were harvested after the respective
104 incubation period using a modified Baermann’s method (MAFF, 1986), and the percentage of eggs
105 that yielded L3 was estimated.

106 Observations on the McMaster isolate were extended to 12 and 23 days to estimate mortality rates
107 at temperatures beyond those typically observed in the field (37°C only) and to examine changes in
108 the proportion of L3 exsheathed over time.

109 *2.4 Statistical analysis*

110 The percentage yield was compared between isolates using a Two-way ANOVA in R (R Core Team,
111 2015). Plots of the residuals were checked for significant departures from normality,
112 heteroscedasticity and influential data points. The expected development success was simulated for

113 each temperature using the GLOWORM-FL *H. contortus* model (Rose et al., 2015). Spearman's rank
114 correlation was used to compare model predictions with observed L3 yield for all isolates combined.

115 The mortality rate of L3 at 37°C was estimated for the McMaster isolate using the proportion
116 surviving the 18 day period between 5 and 23 days incubation: $-\ln(\textit{proportion surviving})/18$. The
117 decrease in numbers of L3 in faeces over time at 37°C was then simulated using the GLOWORM-FL
118 model and either the mortality rate estimated in this study and the mortality rate defined by Rose et
119 al. (2015) to examine the impact of variability in mortality rates between isolates on numbers of L3.

120

121 **3. Results**

122 Mean egg counts (S.D.) on day 0 were 8374 epg (119.6; MHco3(ISE)), 934 epg (125.8; HC1CH), and
123 6358 epg (1253.6; McMaster). All eggs were unembryonated at the time of egg counting.

124 The number of larvae recovered was significantly greater than the egg counts obtained by modified
125 McMaster's technique. Egg counts were therefore corrected based on a recovery efficiency of 40%
126 (Table 1; Morgan, E. R. unpublished data). There was no significant difference in L3 yield (Figure 2)
127 between isolates ($F_{2,58} = 0.645$, $MSE = 537$, $p = 0.528$) and there was no interaction between isolates
128 and temperature ($F_{2,58} = 0.636$, $MSE = 529$, $p = 0.533$).

129 There were apparent departures in observed L3 yield from simulated L3 yield (Figure 2). Observed L3
130 yield was lower than simulated for all three isolates at 37°C, and a higher L3 yield than simulated
131 was observed for the HC1CH at 25°C. However, observed and simulated L3 yield were positively
132 correlated (Spearman's $\rho = 0.66$, $S = 14992.44$, $p < 0.001$).

133 Mortality of the McMaster isolate was rapid at 37°C and a mean of 75% of L3 recovered on day 23
134 were exsheathed (Table 2). Based on these data, an instantaneous daily mortality rate over days 5-
135 23 of 0.252 was estimated, compared with 0.197 estimated by Rose et al. (2015). Nevertheless,
136 simulations using both mortality rates yielded similar results (Figure 3).

137

138 **4. Discussion**

139 No significant difference was observed in the developmental success of the three isolates of *H.*
140 *contortus* tested in this study, despite their disparate origins (East Africa, Australia and Switzerland)
141 and the potential for high levels of genetic differentiation between isolates (Redman et al., 2008).
142 However, the numbers of L3 recovered from cultures maintained at 37°C appeared to be lower than
143 predicted by the model, suggesting the potential for overestimation of transmission risk at higher
144 temperatures. This is unlikely to be an artefact of the correction for egg recovery during faecal egg
145 counting as this would affect all temperatures equally, resulting in a systematic overestimation by
146 the model and not an overestimate at a single temperature. When simulations using the L3 mortality
147 rates estimated from observations on the McMaster isolate were compared with simulations using
148 the L3 mortality rates defined by Rose et al. (2015), there was little biologically meaningful impact
149 on the predicted numbers of L3 over time, suggesting that the reduced recovery rate of L3 from
150 faeces incubated at 37°C compared with the numbers expected from simulations may be due to an
151 increase in the mortality of eggs and/or pre-infective larvae. This is unlikely to affect model
152 simulations using current temperate climatic conditions and no significant difference was found
153 between isolates and the GLOWORM-FL model simulations in this study. However, when the model
154 is applied to scenarios where extreme high temperatures are predicted e.g. some future climate
155 projections or in equatorial regions, the cumulative impact of these small variations may be
156 significant, and additional model validation may be required.

157 The GLOWORM-FL model was parameterised using data from a number of sources where possible to
158 capture variation between isolates (Rose et al., 2015). However, mortality rates of eggs and pre-
159 infective larvae in the GLOWORM-FL *H. contortus* model were based on relatively few data points.
160 Further data were unavailable, presumably due to the difficulties inherent in disentangling the
161 confounding effects of development to the next life cycle stage and mortality. As a result, only
162 observations at ≤4°C and 45°C were available to estimate these parameters (Rose et al., 2015), and

163 the rate of increase in mortality rates with extreme high temperatures might be underestimated.
164 The results presented here may justify modification of the egg and pre-infective larvae mortality
165 parameters in the GLOWORM-FL *H. contortus* model if this is supported by field validation of the
166 updated model.

167 Troell et al. (2006b) observed a similar response to cold treatment in *H. contortus* L3 from Kenya and
168 Sweden (no significant differences between arrest rates, establishment rates nor pre-patent
169 periods). The authors concluded that “there was limited evidence for adaptations to temperate
170 climatic conditions”. However, under untreated conditions (fresh L3) significantly higher arrest rates
171 and longer pre-patent periods were observed in the Swedish isolate, which would act to stabilise
172 populations in the absence of free-living stages (Gaba and Gourbière, 2008), as is common during
173 the Swedish winter, and one could argue that this is evidence of local adaptation. Therefore, the
174 potential for the degree of local adaptation to vary from trait to trait should be addressed when
175 extrapolating knowledge and models to different regions, for example by conducting additional
176 validation to ensure the response of local populations of parasites is captured by model predictions.
177 Moreover, differences have been observed in the temperature thresholds and rates of egg hatching
178 from *H. contortus* of different geographical origin (Crofton and Whitlock, 1965; Crofton et al, 1965).
179 More comprehensive data on parasite responses across a broader temperature range would be
180 useful, but are difficult to obtain, especially from field populations, which are usually of mixed
181 species composition.

182 Finally, changes in selection pressures under future climate change scenarios and adaptation and
183 evolution of parasite populations in response to these changes is difficult to incorporate into climate
184 impact simulations and as a result most models make assumptions of no adaptation (Rose et al.,
185 2015; Caminade et al., 2015). However, such assumptions may be quickly invalidated. For example,
186 regional variation in the hatching behaviour of the GIN *Nematodirus battus* has been observed in the
187 UK and may be a target for future selection (van Dijk and Morgan, 2010). Further research is needed

188 to explore trait variation in GINs and identify traits which may be subject to altered selection
189 pressure under climate change scenarios.

190 The *H. contortus* isolates used in this study were all laboratory isolates which may have adapted to
191 laboratory conditions. Of particular relevance to this study is the reduced pressure to achieve
192 efficient transmission that these isolates experience during routine passage, which typically involves
193 culture of faeces containing eggs from donor animals and oral administration of L3 to recipient
194 animals. Furthermore, the relatively constant conditions experienced by these isolates in the
195 laboratory environment may have led to loss of adaptations to local climates. Under these
196 circumstances, the loss of 'expensive' adaptive traits determining L3 fitness such as migration ability
197 (Knapp-Lawitzke et al., submitted) may be seen, and there may be a regression to a mean phenotype
198 that is well adapted to laboratory environments. Additional work on field isolates to further explore
199 the potential impact of phenotypic trait variation could therefore be valuable. However, purified
200 field isolates are both expensive and difficult to obtain, particularly if the aim is to minimise selection
201 pressure (i.e. minimise the number of passages). This may preclude observations on a range of
202 isolates as presented here. Furthermore, significant genetic differentiation has been detected
203 between laboratory isolates from different geographic origins (Redman et al., 2008). Therefore
204 laboratory isolates are a valuable and valid alternative to field isolates.

205 Based on the observations in the present study and previous observations of phenotypic variation in
206 GIN populations (e.g. Troell et al., 2006b), the following recommendations should be implemented
207 where possible, to increase confidence in climate impact modelling of GINs: parameters should be
208 derived from data from multiple field and laboratory isolates to capture variation; models should be
209 validated using field data from several regions with a range of climatic conditions encompassing
210 both extreme high and low temperatures and rainfall to identify areas of uncertainty in the
211 parameter space; parameters should be calibrated to locally adapted populations if data are
212 available and validated using field data from the region of interest; trait variation and the potential

213 for future adaptation should be considered and assumptions of no adaptation made clear when
214 reporting model output.

215

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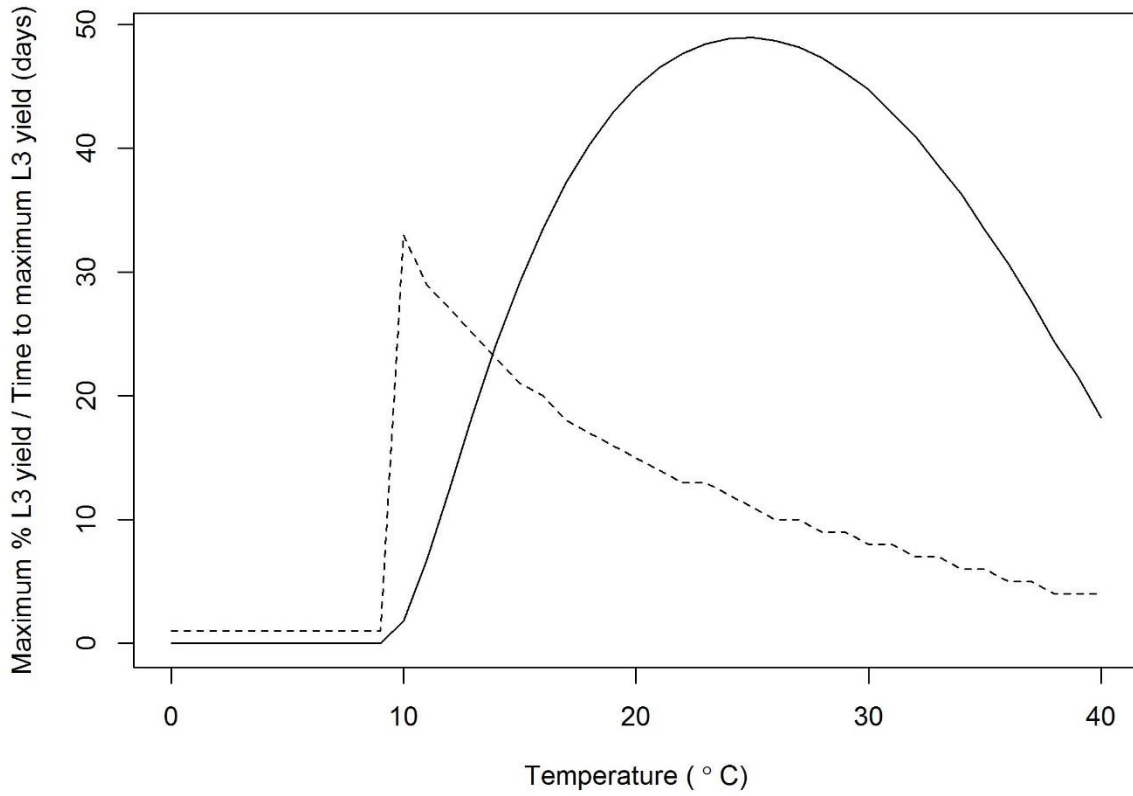
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287 **Figure 1.** Simulated maximum percentage L3 yield (solid line) and time to maximum L3 yield (dashed
288 line) at constant temperatures between 0°C and 40°C.

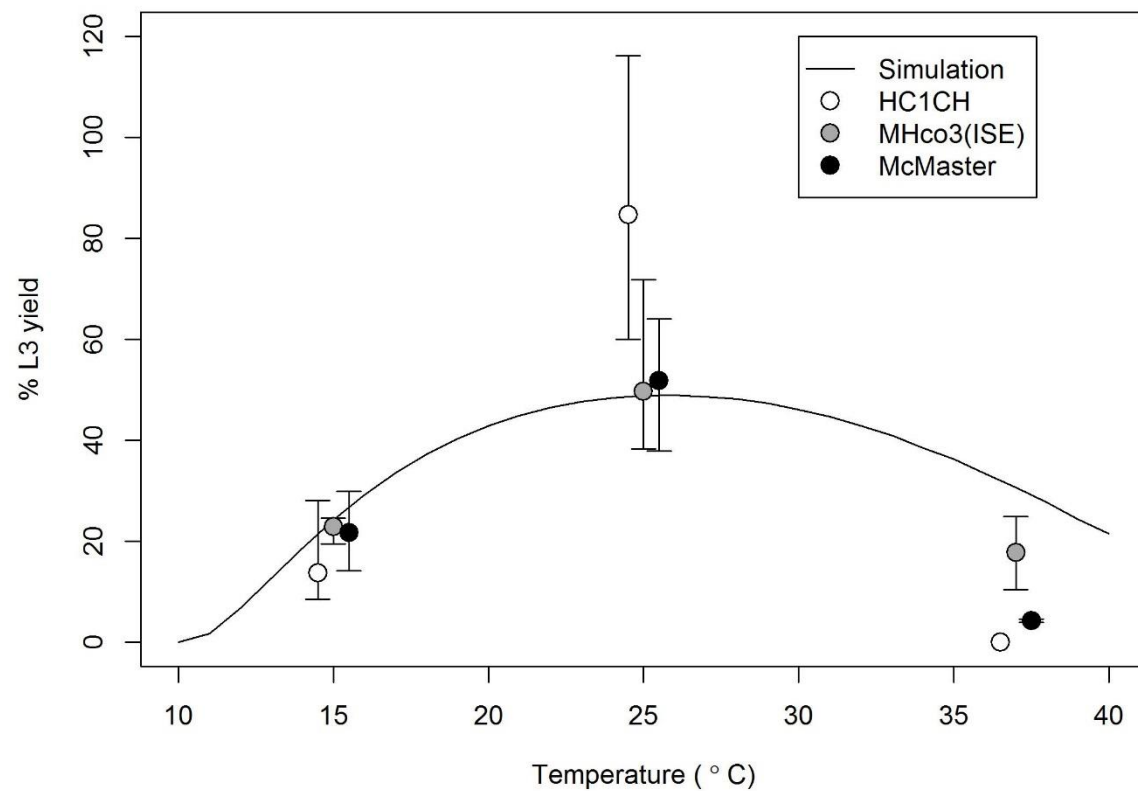


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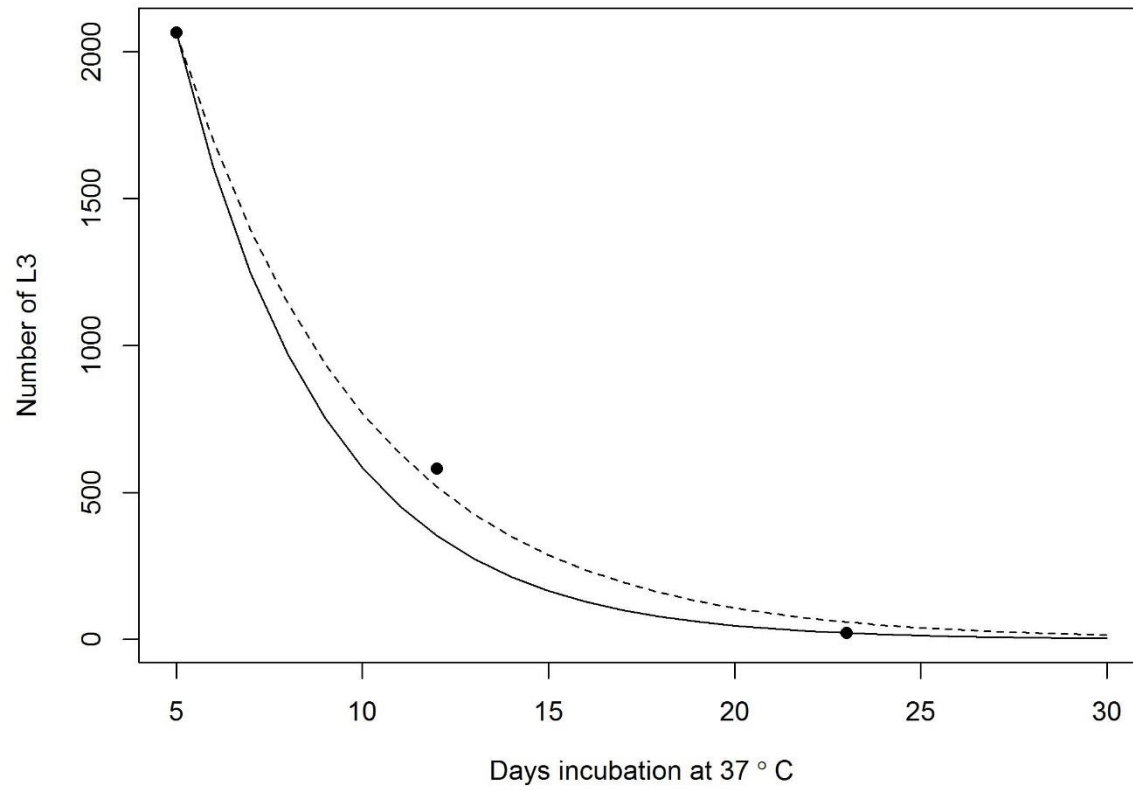
292 **Figure 2.** Mean percentage L3 yield (points) and 95% confidence intervals (whiskers) for *H. contortus* isolates tested at 15°C, 25°C and 37°C (points offset for
293 clarity), and the percentage L3 yield simulated at a range of constant temperatures using the GLOWORM-FL *H. contortus* model (solid line; Rose et al., 2015)



294

295

297 **Figure 3.** Simulated numbers of L3 over time using the mortality rate defined by Rose et al. (2015; dotted line) and the mortality rate estimated using the
298 numbers of McMaster L3 surviving (solid line; Table S2), with the observed numbers of L3 superimposed.



299

300 **Table 1.** Observations of L3 yield in three *H. contortus* isolates and predicted L3 yield based on the GLOWORM-FL *H. contortus* model (Rose et al., 2015). Egg
 301 counts corrected for a 40% recovery efficiency were used to estimate percentage L3 yield.

| Isolate / data source | EPG (S.D.) | Corrected EPG (S.D.) | L3 yield | | | | | | | | |
|------------------------------|-----------------|-------------------------|----------|-----------------|------------|------|----------------|-------------|------|-----------------|------------|
| | | | 15°C | | | 25°C | | | 37°C | | |
| | | | n | LPG (S.D.) | % | n | LPG (S.D.) | % | n | LPG (S.D.) | % |
| MHco3(ISE) | 8374 (199.6) | 20935 (499.1) | 4 | 4791.7 (523.6) | 22.9 (2.5) | 10 | 10400 (2311.3) | 49.7 (11) | 10 | 3715.8 (1012.3) | 17.7 (4.8) |
| HC1CH | 934 (125.8) | 2335 (314.5) | 5 | 320 (214.2) | 13.7 (9.1) | 10 | 1977.5 (478.8) | 84.7 (20.5) | 10 | 0 (0) | 0 (0) |
| McMaster | 6358.3 (1253.6) | 15895.8 (3133.9) | 5 | 3451.1 (1058.2) | 21.7 (6.7) | 5 | 8234 (1685.5) | 51.8 (10.6) | 5 | 688.7 (50.5) | 4.3 (0.3) |
| GLOWORM- FL model | - | - | 1 | - | 24 | 1 | - | 49 | 1 | - | 30 |

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304 **Table 2.** Numbers of *H. contortus* McMaster isolate L3 recovered at intervals from 3g faeces incubated at 37°C, and the percentage of exsheathed L3.

| | 5 days | | | 12 days | | | 23 days | | |
|--------------------|----------------|---------------|--------------|---------------|---------------|--------------|--------------|---------------|--------------|
| | Total L3 | Exsheathed L3 | % exsheathed | Total L3 | Exsheathed L3 | % exsheathed | Total L3 | Exsheathed L3 | % exsheathed |
| Replicate 1 | 2170 | 0 | 0 | 270 | 94 | 34.78 | 0 | NA | NA |
| Replicate 2 | 2150 | 0 | 0 | 210 | 77 | 36.84 | 0 | NA | NA |
| Replicate 3 | 2200 | 0 | 0 | 150 | 30 | 20.00 | 80 | 40 | 50 |
| Replicate 4 | 1950 | 0 | 0 | 100 | 24 | 23.81 | 20 | 20 | 100 |
| Replicate 5 | 1860 | 0 | 0 | 2180 | 569 | 26.09 | 10 | NA | NA |
| Mean | 2066.00 | 0 | 0 | 582.00 | 158.80 | 28.30 | 22.00 | 30.00 | 75.00 |
| S.D. | 151.43 | 0 | 0 | 895.58 | 231.25 | 7.23 | 33.47 | 14.14 | 35.36 |