Implications of phenotypic trait variation for climate change impact modelling of *Haemonchus contortus* populations

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Abstract

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

1. Introduction

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

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

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

2. Methods

2.1 H. contortus *isolates*

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

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

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

2.3 Experiment design

Faeces were mixed thoroughly and a minimum of 3 egg counts conducted using the modified McMaster's method, sensitive to 50 eggs per gram (epg; MAFF, 1986). At the same time, the 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

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

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

3. Results

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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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.

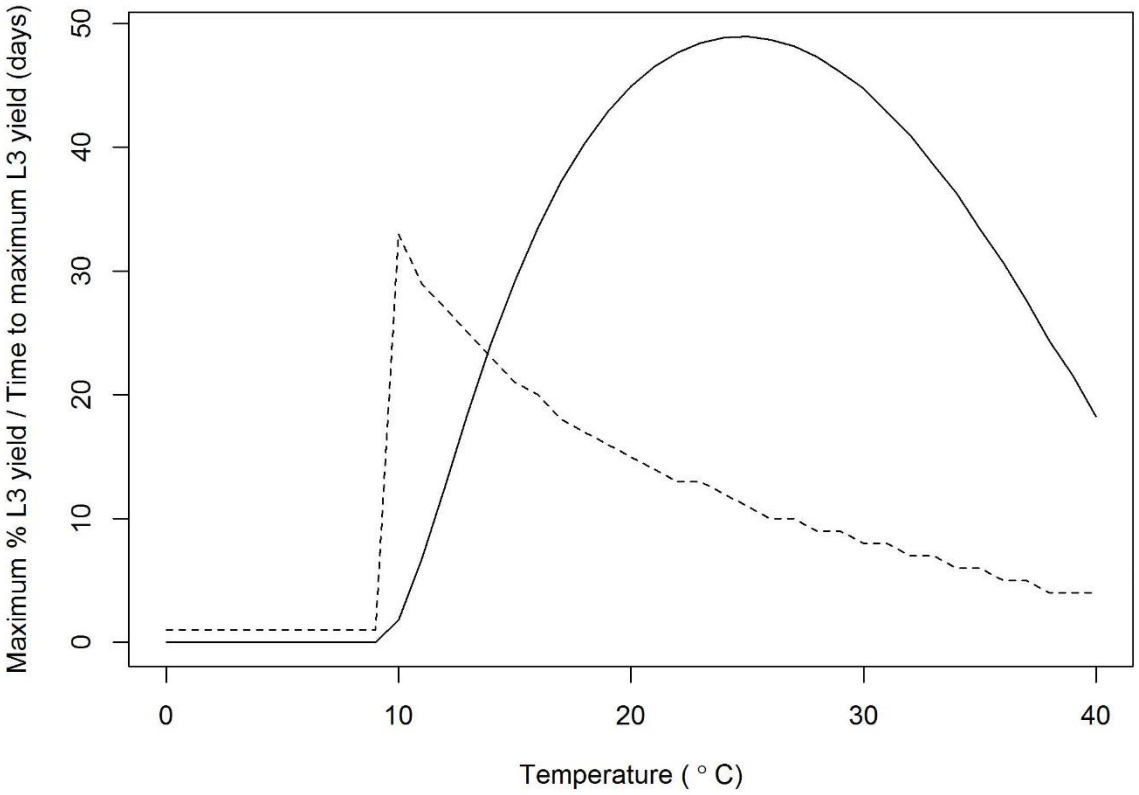

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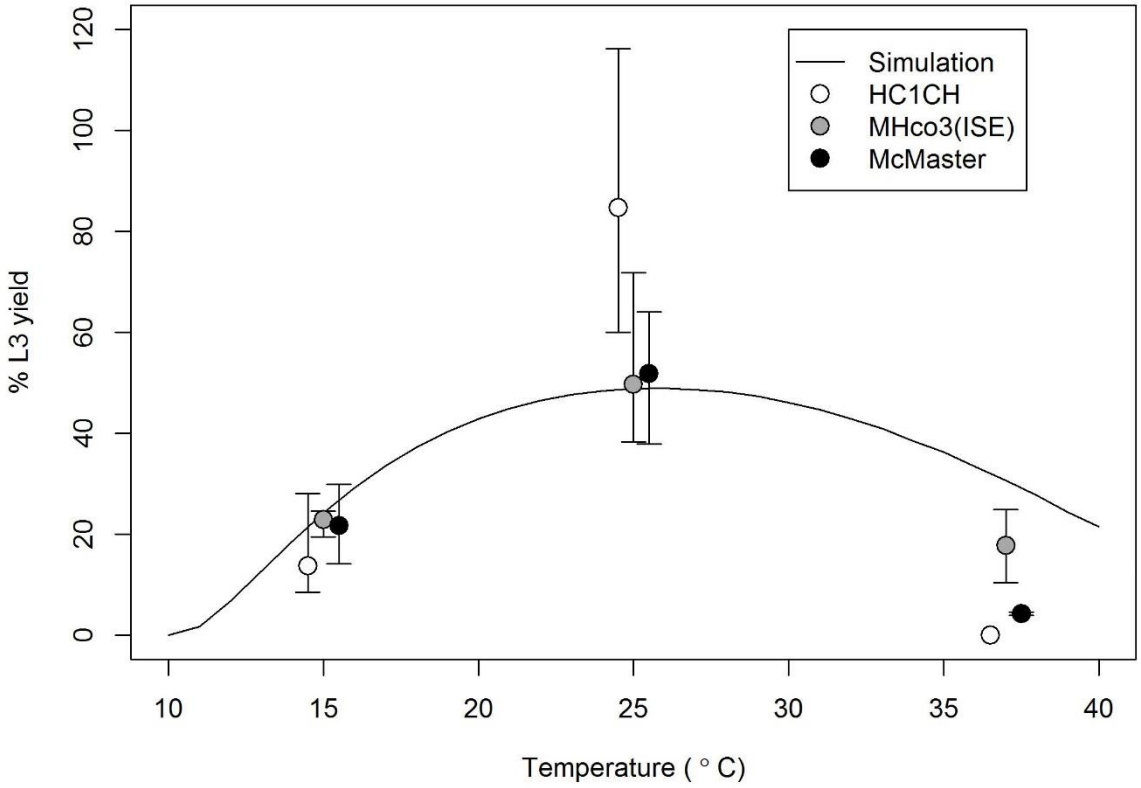
Figure 2. Mean percentage L3 yield (points) and 95% confidence intervals (whiskers) for *H. contortus* isolates tested at 15°C, 25°C and 37°C (points offset for clarity), and the percentage L3 yield simulated at a range of constant temperatures using the GLOWORM-FL *H. contortus* model (solid line; Rose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



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.

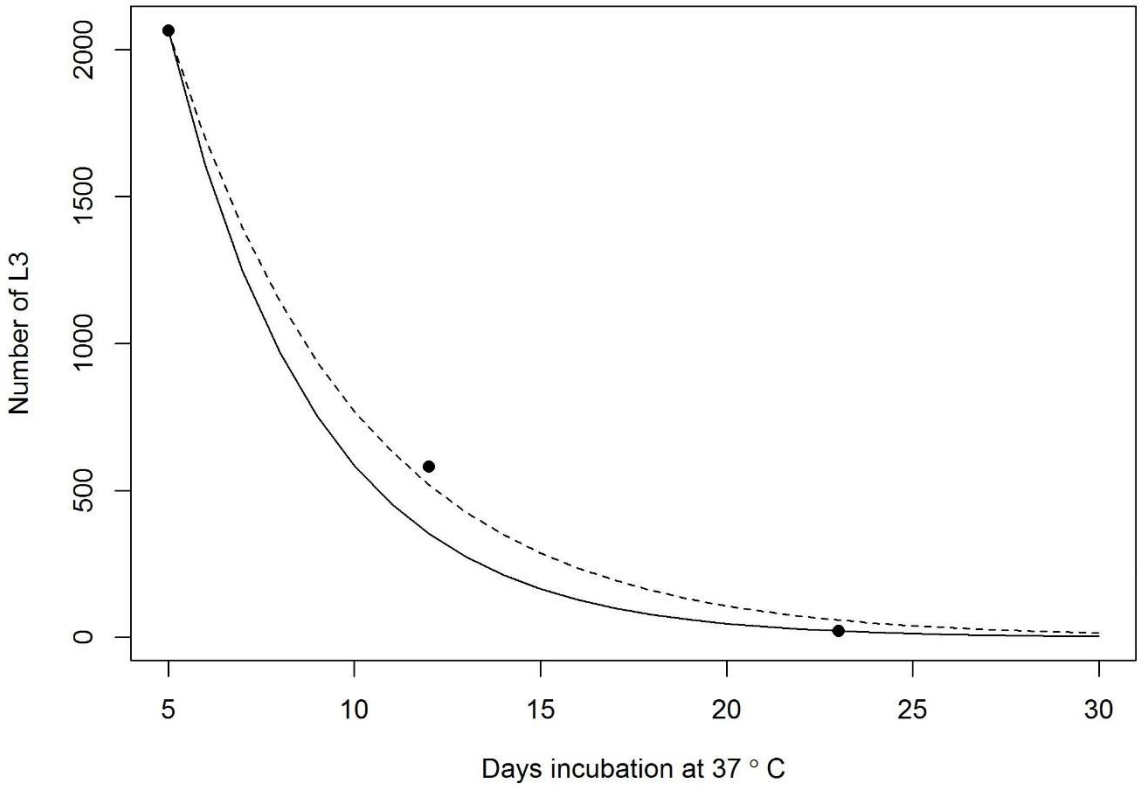


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Table 1. Observations of L3 yield in three *H. contortus* isolates and predicted L3 yield based on the GLOWORM-FL *H. contortus* model (Rose et al., 2015). Egg counts corrected for a 40% recovery efficiency were used to estimate percentage L3 yield.

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

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**

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