**An improved model for the population dynamics of cattle gastrointestinal nematodes on pasture: parameterisation and field validation for Ostertagia ostertagi and Cooperia oncohpora in northern temperate zones**

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**Abstract**

Gastrointestinal nematodes (GIN) are amongst the most important pathogens of grazing ruminants worldwide, resulting in negative impacts on cattle health and production. The dynamics of infection are driven in large part by the influence of climate and weather on free-living stages on pasture, and computer models have been developed to predict infective larval abundance and guide management strategies. Significant uncertainties around key model parameters limits effective application of these models to GIN in cattle, however, and these parameters are difficult to estimate in natural populations of mixed GIN species. In this paper, recent advances in molecular biology, specifically ITS-2 rDNA ‘nemabiome’ metabarcoding, are synthesised with a modern population dynamic model, GLOWORM-FL, to overcome this limitation. Experiments under controlled conditions were used to estimate rainfall constraints on migration of infective L3 larvae out of faeces, and their survival in faeces and soil across a temperature gradient, with nemabiome metabarcoding data permitting species-specific estimates for *Ostertagia ostertagi* and *Cooperia oncophora* in mixed natural populations. Results showed that L3 of both species survived well in faeces and soil between 0 and 30 °C, and required at least 5 mm of rainfall daily to migrate out of faeces, with the proportion migrating increasing with the amount of rainfall. These estimates were applied within the model using weather and grazing data and use to predict patterns of larval availability on pasture on three commercial beef farms in western Canada. The model performed well overall in predicting the observed seasonal patterns but some discrepancies were evident which should guide further iterative improvements in model development and field methods. The model was also applied to illustrate its use in exploring differences in predicted seasonal transmission patterns in different regions. Such predictive modelling can help inform evidence-based parasite control strategies which are increasingly needed due climate change and drug resistance. The work presented here also illustrates the added value of combining molecular biology and population dynamics to advance predictive understanding of parasite infections.

1. **Introduction**

As a major threat to livestock productivity and health (Charlier et al., 2020), in conjunction with the increasing spread of anthelmintic resistance (Kaplan, 2020), more effective and sustainable parasite management strategies are urgently required for gastrointestinal nematode (GIN) infections of cattle. A prerequisite of applying such measures is a good understanding of the epidemiology of GIN in corresponding climatic zones (Hildreth and McKenzie, 2020; Navarre, 2020) to guide empirical control recommendations. Although the intensity of GIN infection in temperate regions is generally lower than in tropical regions (Hildreth & McKenzie, 2020), early and active application of sustainable parasite control strategies is crucial to limit and prevent further development of anthelmintic resistance.

The population dynamics of parasites is subject to complex effects of climate on development, mortality and migration of the free-living stages (Molnár, 2013). Climate change may consequently alter these dynamics with varying effects relative to time and space (Van Dijk et al., 2010; Rose , et al., 2014). Mathematical models of GIN in cattle can provide a framework to incorporate climatic variables into the prediction of current and future infection dynamics (Rose et al., 2015; Rose Vineer, 2020). A robust model framework balances sufficient biological detail with experimentally verifiable parameters (Morgan 2013). Numerous parasite transmission models have been developed in the past several decades addressing various aspects of GIN biology (reviewed by Verschave et al., 2016), and in many cases applied to management scenarios (Rose Vineer, 2020). Because models are parameterised and tested using locally generated data, their applicability in other regions and under climate change scenarios cannot be taken for granted, and should be examined before they are used to guide management decisions.

A model framework has been developed for the free-living stages of GIN (GLOWORM-FL, Rose et al., 2015). It was based on earlier models (Grenfell et al., 1986; Smith, 1990) and integrated the behaviour and ecology of GIN on pasture to predict their climate-dependent seasonal dynamics. The model particularly incorporates the active movement of GIN between substrates (faeces, soil and herbage), in addition to explicitly climate-dependent (i.e., in relation to temperature and rainfall) life history parameters. The model was developed for key GIN species, namely *Haemonchus contortus* and *Teladorsagia circumcincta* in sheep and *Ostertagia ostertagi* in cattle. However, further validation of the *O. ostertagi* model was required and no equivalent model for *Cooperia oncophora* was generated due to insufficient data for parameterisation and validation, despite attempts to fill this gap in recent years (Sauermann and Leathwick 2018). Several limitations hinder the acquisition of data on different life stages of these cattle parasites for model parameterization. Firstly, estimating such parameters experimentally can be challenging, especially for the rainfall-dependent parameters (e.g. migration rate from faeces) as it requires an artificial rainfall simulation system at a scale sufficient to allow replication of multiple bovine faecal pats (Wang et al. 2018). Thus, the better-known parameters are temperature-dependent development and mortality rates, which are more amenable to measurement under controlled laboratory conditions; and rainfall constraints on migration are largely extrapolated from observed thresholds in sheep GIN, which might not be appropriate. Meanwhile, examination of mortality of infective stage larvae (L3) often includes the use of water or other neutral media (e.g. Pandey, 1972; Jasmer et al., 1987) instead of the substrates in which L3 reside in nature, such as faeces and soil. Previous studies have commonly used parasite samples from artificially infected donor animals and focused on the more pathogenic *O. ostertagi*. Consequently, few data are available for *C. oncophora*, despite it being a common and important species in grazing cattle in temperate regions (Avramenko et al., 2017; Wang et al., 2021) and increasingly important due to anthelmintic resistance (Edmonds, et al., 2010; Sutherland and Leathwick, 2011; Geurden et al., 2015). Furthermore, while experimentally passaged parasite strains are valuable and convenient sources of material for experiments, their free-living stages might come to behave differently over time when freed of the requirements of natural transmission on pasture, and it is therefore important to also check the validity of parameters using natural parasite populations (Rose et al., 2016). However, working with natural infections is complicated by the fact that mixed species are usually present, and attributing reaction norms to individual species is impossible without detailed knowledge of species composition at each stage of the experiments.

Some early studies compromised on the complexity of determining species-specific life history parameters by pooling *O. ostertagi* and *C. oncophora* (e.g. Persson, 1974), therefore making the assumption that these species respond in the same way to abiotic factors. However, the validity of this assumption has never been tested. Improvements in nematode identification methods now allow researchers to study species-specific responses in more detail. Nemabiome metabarcoding using deep amplicon sequencing of the ITS-2 rDNA locus is a recently described approach to determine the relative abundance of strongylid nematode species composition in faecal or pasture larval samples (Avramenko et al., 2015; Avramenko et al., 2017; Wang et al., 2020). It has the potential to accurately quantify the species composition at each stage of experiments involving large numbers of samples, so allowing natural infections with natural behaviour to be used for more accurate parameter estimates and field validation.

In this paper, we describe experimental approaches to modify the GLOWORM-FL model parameters for *O. ostertagi* and *C. oncophora* under Canadian climatic conditions. The modified model was then validated with field data collected on three organic cattle farms in western Canada. Finally, the model was applied to several other temperate regions and data from the simulations were used to predict regional differences on pasture L3 availability. Results from simulations such as these can potentially be used to plan grazing strategies for effective parasite management. The approach taken also illustrates the process of adapting existing model frameworks for GIN transmission to new environments, while addressing key uncertainties, and consequently supporting their robust and widespread application to address parasite control in a changing world.

1. **Material & Methods**

2.1 GLOWORM-FL model framework

Detailed illustration of the model framework is described in Rose et al. (2015). In brief, the model framework is based on the lifecycle of free-living stages of GIN (Fig. 1). Starting from the egg stage in faeces, each developmental stage is subject to a stage-specific mortality rate (µi) and L3 in faeces actively migrate onto pasture at a moisture-dependent horizontal migration rate (m1). Once on pasture, L3 are assumed to be reside either in soil (L3s) or on herbage (L3h) with a random bi-directional movement between the two compartments, whose rate depends on temperature and moisture.

2.2 Parameter estimation

The original GLOWORM-FL model was parameterised for three GIN species: *H. contortus, T. circumcincta* and *O. ostertagi* (Rose et al., 2015). It was necessary, however, to make some assumptions to complete the parameterization for *O. ostertagi* due to limited data, and it was not previously possible to parameterise the model for *C. oncophora*. Parameters were prioritised for further experimental evaluation based on three factors: likely importance to model outcomes in temperate areas; paucity of previous data specific to GIN in cattle; and use of parameter values from studies in sheep despite questionable plausibility when applied to cattle, for example the very different structure of the faecal matrix between host species. Thus, the movement of L3 from faeces onto pasture is a key process in the life cycle. Pasture contamination can be altered substantially by the migration of larvae after change of moisture availability, as determined experimentally for *H. contortus* in sheep (Wang et al., 2018). Grønvold and Høghschmidt (1989) reported a constant migration rate of 0.06 for *O. ostertagi* L3 after 1.6 mm simulated rainfall was applied. This finding is useful as point estimation but given the complex matrix of cattle faecal pats, extrapolation of the effect of rainfall variation on L3 migration from this estimate and from studies in sheep is highly uncertain, and more detailed data are needed for cattle. Furthermore, since experiments on *H. contortus* L3 migration showed that rainfall requirement was linked to faecal hydration status (Wang et al., 2014), a single threshold may be unrealistic. Finally, in the original GLOWORM-FL model, the mortality of L3 in faeces and soil were derived from L3 mortality in water. This was due to a lack of direct experimental measurements despite the likely importance of faecal and soil as a reservoir of parasites in dry and cold conditions in temperate regions.

As a consequence of this informal uncertainty analysis, three parameters were selected for *de novo* experimental estimation: L3 larval migration from cattle faeces (*m1*), L3 mortality in faeces (*μ3*), and L3 mortality in soil (*μ4*). Other parameter values were preserved from Rose et al. (2015), and assumed to be identical for *O. ostertagi* and *C. oncophora* pending model validation. Parameters are defined in Table 1.

Parasites used in all experiments were obtained from donor calves artificially infected with mixed GIN L3 obtained from 10 Albertan farms monitored as part of a separate study (Wang et al., 2021). Species composition at each stage of the experiments was quantified by ITS2 nemabiome metabarcoding according to the protocols described by Avramenko et al. (2015) and Wang et al. (2021). Further details of protocols are available at https://www.nemabiome.ca/. All statistical analyses and model simulations were carried out in R Studio (R Core Team, 2021).

* + 1. Experiment 1: Horizontal migration rate from faeces (m1)

The experiment was conducted in a greenhouse at Agriculture and Agri-Food Canada (Lethbridge Research Center) to determine the extent to which free water is necessary for the migration of L3 out of faecal pats and how the migration rate alters with varying amounts of daily rainfall. The greenhouse was heated by hot water and cooled by a series of steps – natural ventilation plus a two-stage evaporative cooling (low and high-speed fans), so the temperature could be maintained at 20 °C. The rainfall simulating system contained 12 irrigation valves (Hunter PGV 1) connected to polypropylene pipes running along the ceiling of the greenhouse. The Argus Controller system permitted the application of varying amounts of simulated rainfall according to the study design.

Faecal samples containing eggs of GIN were thoroughly mixed with a concrete mixer and 54 artificial faecal pats 15 cm in diameter were made by hand. Each pat was placed in a plastic bag with small holes to allow the development to L3 under aeration and optimal moisture conditions. After 3 weeks, artificial pats were placed on 1 mm aperture metal meshes surrounded by an open-ended acrylic cylinder 36 cm in diameter (Additional file 1). Simulated daily rainfall volumes of 0, 1, 5, 10, 15, 30 and 40 mm were applied to experimental pats by the artificial rainfall system for 7 days. Six replicates (experimental pats) were included in each rainfall volume group. After each simulated rainfall event, splashed L3 attached on the cylinder wall were immediately washed into the container positioned below. Once every 24 hours, L3 attached on the metal mesh, due to active migration, were also washed into the container. Daily L3 in the container (splash + active migration) were collected, enumerated, and fixed in 70% ethanol for further DNA analysis. Finally, ITS2 nemabiome metabarcoding (see Avramenko et al., 2015 for detail) was applied to determine the species composition of extra-pat L3 on each day of the trial. The species composition and relative proportion of L3 that had migrated out of faeces was calculated as*: extra-pat L3 / (extra-pat L3 + intra-pat L3)*, corrected for recovery efficiency (see Additional file 2). Regression analysis was then used to estimate the relationship between migration rate and the amount of daily rainfall. Finally, One-way ANCOVA was conducted to compare L3 migration between *O. ostertagi* and *C. oncophora* whilst controlling for rainfall.

* + 1. Experiment 2: L3 mortality rate in faeces (µ3)

To determine the daily mortality of L3 in faeces in relation to ambient temperature, faecal samples containing GIN eggs were thoroughly mixed in a 220-watt hand mixer (Cuisinart HM-70C, Stamford, US) for each temperature group and placed into six-well plates with 3 g aliquot in each well. All samples were cultured at 20°C for 3 weeks and sprayed lightly with water every 2-3 days to maintain moisture, so the L3 stage could be reached. Following development of L3, on Day 0, plates were placed in incubators at the following temperatures: -15 °C, -10 °C, -5 °C, 0 °C, 8 °C, 20 °C and 30 °C with 95% relative humidity (RH). During the 364 days experiment, three replicates were removed from each incubator at predetermined intervals (Additional file 3) and L3 enumerated. ITS2 nemabiome metabarcoding was applied to determine the species composition at each time point for each temperature.

Finally, ITS2 nemabiome metabarcoding was applied to the pooled L3 of three replicates to determine the species composition at each time point. The proportion of L3 surviving at each time point was calculated as: *L3t / L3t0 × 100,* where L3t represents the number of survived L3 at the day of checking and L3t0 represents the number of L3 at Day 0 when it was assumed that no mortality had occurred. The detailed statistical approach to estimate the instantaneous daily rates can be found in Rose et al. (2015). Finally, One-way ANCOVA was conducted to compare L3 mortality in faeces between *O. ostertagi* and *C. oncophora* whilst controlling for temperature.

* + 1. Experiment 3: L3 mortality rate in soil (µ4)

L3 mortality in soil relative to ambient temperature was determined by extracting L3 from three replicates of garden soil (mix of black topsoil, peat and perlite) at predetermined intervals (Additional file 3) incubated at constant temperatures of -15 °C, -5 °C, 5 °C, 20 °C and 30 °C. The soil samples were autoclaved to eliminate free-living nematode. Firstly, 20 g soil sample was distributed into each of 90 petri dishes (5 cm in diameter), representing 90 replicates across all temperatures. Each replicate was seeded with 1000 L3 cultured at 20 °C for 3 weeks using eggs obtained from calves previously infected with field collected L3. Experimental L3 were stored at 4 °C for less than one week before seeding. To recover L3 at each time point, the soil sample was soaked in a beaker with 300 ml tap water for 10 minutes. The water was then thoroughly stirred to resuspend the L3 in the beaker. After waiting for 30 seconds, the heavy debris sedimented to the bottom, leaving the suspension and L3 relatively clean. L3 were then extracted using a sieving and flotation technique described in Wang et al. (2020) before enumeration and ITS2 nemabiome metabarcoding. The same statistical methodology as in section 2.2.2 was used to estimate the instantaneous daily rates.

2.3 Model validation

Field observations of pasture L3 populations in the absence of cattle grazing were used to validate the predicted trajectory of L3 abundance for *O. ostertagi* and *C. oncophora* during the 2019 grazing season. A detailed description of the field study used to generate the data can be found in Wang et al. (2021). In brief, grass samples were collected every three weeks from three organic beef cattle farms located in Red Deer, Castor and Waterton (Alberta, Canada). After grazing on one pasture for 2-9 days, the herd moved to the next pasture, without returning for the remainder of the grazing season. This management scheme allowed the development of free-living stages from eggs in faeces to L3 on herbage, in the absence of further egg deposition or L3 offtake through grazing, so that pasture L3 availability can be monitored every three weeks for the rest of the grazing season. After each sampling event, the total number of L3 on grass around each faecal pat (which had migrated out from the faeces) was determined from grass L3 count. Total L3 numbers were then converted to number of each individual GIN species based on the relative abundance data derived from ITS2 nemabiome metabarcoding. One solar-powered weather station (HOBO RX3000, Onset Corp, MA, USA) was set up for each farm, with probes to record temperature (°C) at 1 cm above ground level and precipitation (mm) every 15 mins. Recorded data were transferred daily to HOBOlink web-based software (https://www.hobolink.com/) where the latest data were downloaded.

Model validation was conducted for *O. ostertagi* and *C. oncophora* separately, which was possible due to specific identification of recovered L3 using ITS2 nemabiome metabarcoding. The initial number of eggs per faecal pat for each pasture was obtained from the mean of 20 individual fresh faecal samples collected at the beginning of the study while all other initial values in the model were set to 0. The eggs therefore seeded the GLOWORM-FL model, which then predicted the abundance of subsequent free-living stages as a function of the weather. The simulated values were first compared with the field observation at the corresponding time point, using pooled data from all pastures to maximize generality and statistical power. The same approach was then applied to each pasture individually. Model goodness of fit was estimated using a linear regression through the origin of the observed and simulated pasture L3 as described by Rose et al. (2015), following establishment of a significant correlation. A statistically significant regression with low residual error was taken to indicate that the model adequately predicts the observed pasture L3. Model performance (compared to field observation) was estimated by the slope of the regression through the origin. If the slope was not significantly different from 1, the model was able to reproduce the magnitude of the observed pasture L3, with values significantly less than 1 indicating overestimation of pasture L3 level by the model (or underestimation by the larval recovery method) and values significantly greater than 1 indicating underestimation of pasture L3 level by the model (or overestimation by the larval recovery method)

Linear regression was thus used synoptically to indicate quality of overall fit and direction of error, rather than appraisal of extraneous variation in the data set: hence, non-independence of data points within pastures and within time points, was not taken into account. This is justified on the basis that the outcome of interest is whether, within and across pastures, the model is able to predict relative changes in L3 abundance, rather than to determine formally which factors other than the model (e.g. individual pasture, sampling date) have a significant bearing on observed L3 levels.

2.4 Model application: Can the model inform strategies for pasture rotation under climate variation?

The *O. ostertagi* model was used for risk assessments due to the higher pathogenicity of *O. ostertagi* relative to *C. oncophora* (Charlier et al., 2020).The model was applied to investigate the effect of grazing season on pasture L3 availability, alongside interannual variability and the effect of varying climatic regions. Firstly, five years of weather data (2015-2019, see Additional file 4 for the raw data) from four Canadian and three European regions were obtained from Meteorological Offices (gov.ca and NOAA). These areas represented a range of temperate regions in the northern hemisphere. The model was seeded with 1000 *O. ostertagi* eggs (egg deposition at Day 1) and run for six weeks, starting in each month (from January to December) for all seven locations and five years (2015 - 2019). The precise number of eggs used to seed pasture is not important, as the outcome of interest was the relative differences in predicted patterns of pasture contamination between years and regions. For each of these simulations, cumulative exposure to L3 on herbage following egg deposition was extracted as the dependent variable. This indicates the consequence of egg shedding for pasture contamination over a 6-week grazing period following the start of egg deposition (Wang et al., 2021). A generalized linear mixed model (GLMM) was then fitted to the generated dataset with ‘month of egg deposition’ as a fixed effect, and ‘location’ and ‘year’ as random effects. The month (season) in which egg deposition provides the highest risk for grazing cattle was thus identified along with the variation in seasonal risk between locations and years. The purpose of this analysis was to identify whether the refined model can be used to identify differences in seasonal epidemiology between regions within a similar climatic zone, which could help to guide strategic decisions on grazing and parasite management.

1. **Results**
   1. Model parameterization

The model was parameterized for two cattle GIN species: *O. ostertagi* and *C. oncophora* (Fig. 2-4, Table 2).

* + 1. Experiment 1: Horizontal migration rate of L3 (m1)

To estimate the instantaneous daily migration rate of *O. ostertagi* and *C. oncophora* out of faeces, linear regression models were fitted to the proportion of daily migrated L3 for each species across a range of daily rainfall volumes (Fig. 2). For both species, migration rate increased with increasing daily rainfall (R2adj = 0.58-0.72, P<0.001) in an approximately linear fashion. Although very little migration occurred below 15 mm rainfall and linear regression underestimated migration at 30 mm daily simulated rainfall for *C. oncophora*, the data did not enable more complex relationships to be parameterised and so linear regression equations were used as model parameters for *O. ostertagi* and *C. oncophora*. Regression of migration rate against daily rainfall predicted a minimum rainfall threshold for migration of 5.8 mm and 6.7 mm for *O. ostertagi* and *C. oncophora,* respectively (Fig. 2). One-way ANCOVA was conducted to compare L3 migration rate of *O. ostertagi* and *C. oncophora* whilst controlling for rainfall. *C. oncophora* L3 had a significantly higher migration rate than *O. ostertagi* (F1, 67 = 7.41, P = 0.008).

* + 1. Experiment 2: L3 mortality rate in faeces and soil (µ3 and µ4)

Temperature-dependent daily L3 mortality rates in faeces were highest at extremely high and low temperatures. Therefore, two polynomial models were fitted to the log-transformed instantaneous mortality rates for *O. ostertagi* and *C. oncophora,* respectively (Fig 3: mortality rate in faeces; Fig. 4: mortality rate in soil). Mortality rate at 40 °C was set at 1 based on Pandey (1972). One-way ANCOVA was conducted to compare the L3 mortality rate of *O. ostertagi* and *C. oncophora* whilst controlling for temperature. For *O. ostertagi*, no significant difference was detected for L3 mortality in faeces versus soil (F1, 270 = 1.86, P = 0.173). On the other hand, *C. oncophora* L3 had significantly higher mortality in faeces than in soil (F1, 270 = 6.26, P = 0.013). For both species and matrices, L3 mortality rate was low between 0 and 30 °C, and similar between species (Fig. 3 and 4, Table 2).

3.2 Model validation

The range of mean daily ground temperature and daily precipitation during the field observation period was 1 – 25 °C and 0 – 28 mm, respectively. The model performed reasonably well when tested against data collected on three Albertan farms during the 2019 grazing season. Fig. 5 shows the validation statistics of pooled observed and simulated values of L3 per pat from all pastures for *O. ostertagi* and *C. oncophora*. Fig. 6 and Fig. 7 show the validation statistics of observed and simulated values of L3 on herbage per pat from individual pastures, for each examined species. The abundance of L3 in field samples reflected the model predictions. Overall, in the aggregated dataset from all pastures, the model predicted 78% of observed variation in *O. Ostertagi* L3 counts (R2 0.782, P<0.001) and 77% for *C. oncophora* (R2 0.775, P<0.001). A lower R2 value of 0.68 was observed without separating the species using ITS2 nemabiome metabarcoding. In both cases, however, the fitted slope was less than one, indicating that the model predicted higher L3 abundance than was observed at higher pasture contamination levels, and lower levels than observed at lower pasture contamination levels. On each of the eight pastures, herbage L3 count in the three to four months grazing window were used as field observations. The performance of the model was similar for the two GIN species investigated.

Considering individual pastures, observed L3 levels were significantly correlated with predicted L3 abundance on herbage at the corresponding time point for seven of the eight trial pastures, for each species. The model predicted 47-84% of the variation in observed L3 over time for *O. ostertagi* (Fig. 6), and 37-83% for *C. oncophora* (Fig. 7). One field, Castor Pasture 3, was predicted to have relatively low levels of L3 over the grazing season, and neither the *O. ostertagi* nor the *C. oncophora* model were able to predict temporal variation in observed L3 levels, which were low and varied widely between sampling points.

For four out of eight of the pastures the *O. ostertagi* model predictions were a good fit to the observed L3, with significant correspondence between observed and predicted data, and a predicted slope of around 1 (Red Deer Pasture 3, Waterton Pasture 2, Castor Pasture 1, Castor Pasture 2; Fig. 6). On other pastures the relationship between observed and predicted L3 was significant, indicating that the model replicated seasonal patterns of pasture contamination, but the model appeared to systematically over-predict (Red Deer Pasture 1, Red Deer Pasture 2) or under-predict (Waterton Pasture 3, Castor Pasture 3) observed L3 counts. On these latter pastures, underprediction tended to be more pronounced later in the grazing season, whereas qualitatively, the model appeared to correspond well with observed L3 earlier in the season. The *C. oncophora* model had a greater tendency to over-predict L3 density on pasture, as indicated by the estimated slopes of the linear regressions (Fig. 7; Red Deer Pasture 1 and 2, Waterton Pasture 2). However, based on qualitative assessment, the model performed well for four out of eight of the pastures (Red Deer Pasture 1, Castor Pasture 1, Castor Pasture 2, Waterton Pasture 3), and for other pastures the seasonal pattern of pasture contamination was replicated, albeit not the magnitude of L3 on pasture.

* 1. Model application: Can the model inform strategies for pasture management under climate variation?

The effects of grazing season, year and location on predicted pasture L3 availability during 6-week pasture residence periods are illustrated for the seven northern temperate regions in Fig. 8. GLMM and Tukey’s post hoc test on the combined data for all regions showed that cumulative pasture L3 is the highest if the 6-week grazing period starts in July, followed by June and August. This varied significantly by region (P < 0.001) but not between years (P = 1). This indicates a consistent seasonal pattern in L3 development potential on temperate pastures, which across regions tends to produce the highest L3 abundance during a 6-week pasture gazing period starting between June and August. Simulations tracking L3 numbers over 4 and 9 weeks following the start of grazing produced different absolute abundance, but the same qualitative patterns described here (data not shown).

Model simulations showed differences between locations in overall L3 yield, the duration of the effective transmission season, and the individual months during which pasture contamination with eggs translates most efficiently to L3 availability. Thus, both overall L3 abundance and the duration of the transmission season were greatest for Ontario. By comparison, the European locations had a slightly shorter predicted transmission season and a much lower overall larval yield. The three locations in Alberta were characterised by an even shorter transmission season but an intermediate larval yield, i.e., a more sudden transition between winter conditions (unsuitable for L3 development) and suitable summer conditions.

The month most suitable for larval development varied somewhat between locations, with August showing the highest L3 yield in Norway, and June showing the highest L3 yield in Ontario, for example. The consequences of egg output for pasture contamination with L3 in the following weeks therefore differ across the transmission season, and the months with the greatest potential for transmission vary between locations.

1. **Discussion**

The model presented in this paper was adapted for *O. ostertagi* and *C. oncophora* based on the original framework (Rose et al., 2015) by estimating key parameters for these species and thus improving the model applicability to cattle trichostrongylid nematodes. ITS2 nemabiome metabarcoding has recently been applied to epidemiological studies of cattle GIN in western Canada (Wang et al., 2020; Wang et al., 2021) and the current study demonstrated for the first time the power of this species identification tool to support the development and testing of predictive models. The application of ITS2 nemabiome metabarcoding allowed parameter estimation for multiple GIN species using material recovered from natural field samples; and testing of model predictions against observed natural mixed GIN populations. This approach has three major advantages: first, it eliminates the potential behavioural bias arising from multiple generations of laboratory passage. Secondly, it substantially increases the efficiency of model parameterization experiments as each species would otherwise require its own set of experiments using mono-cultured laboratory strains. Thirdly, it allows models to be tested using mixed infections typical of field data. The model validation results confirmed the value of using species-specific parameter estimates, which improved the correlation between model predictions and observed field data.

The most efficient way to estimate model parameters is by analysing data from previous relevant studies (e.g. Verschave et al., 2014). Unfortunately, for the purposes of model parameterisation gaps in the literature occur due to the varying aims and methods between studies and the complexity of the GIN lifecycle. It is possible to address some of these gaps using laboratory based experiments. For example, Wang et al. (2014) identified that a lower rainfall threshold of 2 mm was necessary for the migration of *H. contortus* L3 out of sheep faeces, whilst increasing rainfall above this level did not further increase the rate of migration - an experiment which would have been difficult to control under field conditions. Similar data were not available for the translocation of cattle GIN onto pasture, aside from a single study demonstrating horizontal migration of GIN from cow pats following 1.6 mm of rainfall Grønvold and Høghschmidt (1989). This study aimed to improve the L3 horizontal migration rate (m1) parameter in the GLOWORM-FL model by assessing varying levels of simulated rainfall applied to artificial cattle dung pats containing a mixed parasite population. Unlike horizontal migration of GIN L3 from sheep faeces (Wang et al., 2014), linear regression analysis suggested a positive linear relationship between rainfall and L3 migration from cattle dung. The lower daily rainfall threshold for L3 migration was around 5 mm, thus higher than for sheep, and this might be explained by the tendency of cowpats to desiccate from the outside, forming a crust that must be moistened before L3 can migrate across it. It remains unclear if the migration pattern is sigmoidal because 40 mm was the highest volume of simulated rainfall investigated in this study, but this might have limited practical relevance since the highest daily rainfall in the field study was 28 mm. One limitation of the current study was that the larval migration experiments used dung pats that were moist to begin with. Further work will be required to determine the lower rainfall threshold for *O. ostertagi* and *C. oncophora* L3 migration from faeces under varying starting faecal moisture levels, and if migration saturates beyond 40 mm daily rainfall. In the field, delayed L3 migration from faeces in dry weather, along with their survival within the faecal pat (see below), could lead to sudden increases in L3 abundance on pasture following drought-breaking summer rainfall. The data presented here equip the GLOWORM-FL model to predict the circumstances in which this might occur and its epidemiological importance.

Other key model parameters assessed in the current study were L3 mortality rates in faeces and soil. These parameters were selected for further investigation as they directly determine the longevity of pasture infectivity, and existing estimates were based on very limited studies and extrapolated between water, soil and faeces (Rose et al., 2015). Results of the present study suggest little difference in L3 mortality rate between *O. ostertagi* and *C. oncophora*, either in faeces or in soil. Survival of L3 was high in both compartments across a wide temperature range, suggesting that dung pats and soil can provide effective reservoirs of infective larvae, and supporting results of Wang et al., (2021) whereby L3 remained in dung pats throughout the grazing season. For cattle GIN, there appears to be little justification for inflating mortality rates in faeces relative to soil, as suggested by Rose et al. (2015). In contrast to mortality rates, *C. oncophora* was found to have a significantly higher horizontal migration rate than *O. ostertagi*. These results may indicate similar thermal tolerance but differences in moisture-dependant larval activity between these two species. The updated parameter estimates described provide the opportunity to predict the effects of climate and climate change on infection dynamics more robustly. The use of ITS2 nemabiome metabarcoding made it possible to get more accurate species-specific information from experiments on natural mixed species populations.

Any well-developed predictive model has limited credibility unless tested with field data. Model validation, however, can be challenging due to the complexity of factors under field conditions that increase the gap between controlled laboratory conditions and natural environments. A robust validation requires enough study power (e.g. sample size) without excessive bias, which means a large scale field-setup is often required. In this study, model performance was tested using data collected from a total of eight pastures on three farms. The species-specific models were first validated with pooled data from all eight pastures whilst ITS2 nemabiome metabarcoding allowed both the *O. ostertagi* and *C. oncophora* models to be tested using the same set of field samples. The results confirmed the added value of nemabiome data, with a higher adjusted R2 value for the species-specific models (0.78 and 0.77) than the non-species-specific model (0.68). A statistically significant relationship between predicted and observed L3 on seven out of the eight pastures indicates that the model was able to effectively predict the seasonal patterns of development and survival. The model also effectively predicted the magnitude of pasture contamination on 4 out of 8 of the pastures. On the remaining pastures, the model both overpredicted and underpredicted L3 densities, indicating no systematic model bias towards over- or underprediction. In the cases of overprediction, this tended to take place later in the season, which may suggest that survival of L3 on pasture requires further calibration. Factors such as input faecal egg counts, unexplained biological variation and measurement error may affect agreement between model prediction and field observations. In particular, measurement error is more likely when L3 levels are numerically low and its effects will be more visible (e.g. Red Deer Pasture 2 and Castor Pasture 3). Pastures may also differ, and vary over time, in unmeasured factors that potentially affect L3 abundance and/or recovery, including characteristics of soil (physics-chemical and biotic), herbage (sward height, botanical composition) and weather (wind, dew). Variation in larval recovery from herbage might also be density-dependent, with sedimentation impeded by high larval densities, which might explain the shallow regression slope between predicted and observed L3 density. A balance between model complexity and feasibility was achieved which means more complicated scenarios such as more intermittent application of simulated rainfall and mechanical spreading of larvae by cattle movement. These factors will be useful to be implemented to the updated version in the future. Overall, however, the model performed well in reproducing the observed seasonal patterns in the northern continental climatic zone of western Canada.

To further explore larval dynamics in temperate regions, the model was used to predict the availability of L3 to grazing ruminants, as a function of egg output onto pasture and the weather. The relative abundance of *O. ostertagi* L3 on pasture subsequent to grazing in different months was simulated in seven regions (four in Canada and three in Europe) and over five years (2015-2019). Results showed that L3 levels accumulating on pasture in the six weeks following the start of egg deposition were highest in June-August, with some differences between regions. Relative to sites in Alberta, the longer season transmission and higher potential L3 yield in southern Ontario suggests that osteragiosis could present a larger risk to cattle in this area, and that evasive grazing regimes or suppressive treatment should be considered earlier in the calendar year. In Scandinavia, on the other hand, infection pressure is likely to be lower overall but conditions for transmission are relatively high in later months, and consequently producers should be wary of the potential for late-season L3 contamination from animals administered anthelmintics soon after turnout. In Alberta, contamination of pastures with GIN eggs can produce L3 between May and September, but yields are highest in June and July, and this period should be prioritised when considering treatment or evasive grazing plans. The model concurs with previous empirical results (Wang et al., 2021) that rotational grazing using a maximum 6-week residency time is a sensible approach to limiting GIN exposure in this region, provided sufficient clean grazing is available to ensure adequate pasture resting before return to grazing. Overall, the results indicate that while a similar seasonal pattern generally exists across temperate regions, differences can be observed at a finer temporal scale (e.g. monthly) owing to varying local climatic conditions. Inter-annual differences were modest, suggesting that management strategies that are well-adapted to local conditions can be developed and applied consistently. The model presented here could be used to explore in more detail the implications of different grazing and treatment strategies for parasite challenge in cattle in temperate regions, both in general and adapted to specific locations.

1. **Conclusion**

This study improves the GLOWORM-FL model for cattle GIN by further developing the parameters for *O. ostertagi* and *C. oncophora* larval mortality and migration dynamics. The updated model parameters were tested with weather data from the northern continental climatic zone of western Canada, against observed pasture larvae counts. Finally, the model was applied to a number of additional Canadian and northern European regions to explore the implications of climatic variation for larval dynamics and potentially for management strategies. This stepwise, systematic approach builds the possibilities of applying the model more widely in temperate areas, and to devise and optimise specific interventions. Crucially, the synthesis of predictive computational modelling and molecular biology, through integration of ITS2 nemabiome metabarcoding with GLOWORM-FL, offers clear potential for better understanding of parasite dynamics at pasture and for devising robust evidence-based strategies for GIN control under current and future conditions.

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Fig. 1. GLOWORM-FL model framework. Parameters and stage of lifecycle (state variables) are explained in Table 1. Adapted from Rose et al. (2015).

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Fig. 2. Instantaneous daily horizontal migration rate (m1) of L3 from faeces onto pasture for O. ostertagi (panel a; points) and C. oncophora (panel b; points) across a range of experimentally simulated rainfall levels; Statistical output for the regression model (abline) is provided in Table 2.

Chart, scatter chart

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a)

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b)

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b)

a)

Chart, histogram

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a)

Chart

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b)

Fig. 5. Model validation on aggregated L3 count data from all pastures. The diagonal line indicates hypothetical perfect fit between the observed and simulated herbage L3 count (slope = 1). O. ostertagi results (panel a): P < 0.001, R2 (R2 adj) = 0.782 (0.780), Slope (95% CI) = 0.834 (0.764 – 0.904). C. oncophora results (panel b): P < 0.001, R2 (R2 adj) = 0.775 (0.774), Slope (95% CI) = 0.813 (0.779 – 0.847).

Chart, scatter chart

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Chart, scatter chart

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Diagram

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Fig. 8. Model projection of the cumulative number of L3 on pasture following a 6-week grazing period by infected cattle starting in each month of the year in seven temperate regions. Each month of the year was set as the starting time of the simulations, and the graph includes L3 appearing on pasture in the first half of the following month. Error bars represent the standard deviation from 5 years’ data. The *O. ostertagi* model only was used in this part of study.

Timeline

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Table 1. State variables and parameters used in the parasite dynamic model, after Rose et al. (2015). Shaded parameters were prioritised for experimental estimation, see text.

|  |  |  |
| --- | --- | --- |
| State variable (life cycle stage) | Definition |  |
| E | Eggs | - |
| L | Pre-infective stages (L1 and L2) larvae | - |
| L3f | Infective stage larvae (L3) in faeces | - |
| L3p | Total L3 on pasture (soil + herbage) | - |
| L3h | L3 on herbage | - |
| L3s | L3 in soil | - |
| Parameter | **Definition** | **Units** |
| *δ* | Development rate from egg to L3 | Instantaneous daily rate |
| *m1* | Horizontal migration of L3 onto pasture | Instantaneous daily rate |
| *m2* | Proportion of total pasture L3 on herbage | Proportion |
| *µ1* | Egg mortality rate | Instantaneous daily rate |
| *µ2* | L1 and L2 mortality rate | Instantaneous daily rate |
| *µ3* | L3 mortality rate in faeces | Instantaneous daily rate |
| *µ4* | L3 mortality rate in soil | Instantaneous daily rate |
| *µ5* | L3 mortality rate on herbage | Instantaneous daily rate |

Table 2. Parameter estimates obtained from laboratory experiments. ANCOVA results show the comparison between the two species. *µ3* vs*µ4* at the bottom compared the mortality rate. Oo: *Ostertagia ostertagi*, Co: *Cooperia oncophora*. The source of the parameter estimates: Rose et al. 2015, Experiment 1 (this study), Experiment 2 (this study). The rest of model parameters are the same to the original GLOWORM-FL model and can be found in Rose et al. 2015.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Species | Estimate | 95% CI (low, high) | ANCOVA |
| *m1* | *Oo* | R2 = 0.59, R2adj = 0.58, F1, 33 = 48.29, p < 0.001; m1 = 0.0066 × Rainfall - 0.0382 | Slope: 0.005 – 0.009  y-intercept: -0.080 – 0.003 | F1, 67 = 7.41,  P = 0.008 |
| *Co* | R2 = 0.73, R2adj = 0.72, F1, 33 = 89.85, p < 0.001;  m1 = 0.0106 × Rainfall - 0.0711 | Slope: 0.008 – 0.013  y-intercept: -0.120 – -0.022 |
| *µ3* | *Oo* | R2 = 0.92, R2adj = 0.91, F3, 20 = 78.1, p < 0.001; µ3 =exp(-4.864 – 0.206 × Temp + 0.0048 × Temp2 + 0.00008 × Temp3) | Temp: -0.2364, -0.1748  Temp2: 0.0021, 0.0075  Temp3: 0.00001, 0.00015  y-intercept: -5.3416, -4.3864 | F1, 369 = 0.10,  P = 0.747 |
| *Co* | R2 = 0.93, R2adj = 0.92, F3, 20 = 89.15, p < 0.001;  µ3 =exp(-4.726 – 0.204 × Temp + 0.0044 × Temp2 + 0.00009 × Temp3) | Temp: -0.2326, -0.1758  Temp2: 0.0019, 0.0069  Temp3: 0.00003, 0.00015  y-intercept: -5.1676, -4.2844 |
| *µ4* | *Oo* | R2 = 0.98, R2adj = 0.97, F3, 14 = 192.2, p < 0.001; µ4 =exp(-5.743– 0.1755 × Temp + 0.0068 × Temp2 + 0.00003 × Temp3 | Temp: -0.1957 – -0.1553  Temp2: 0.0051 – 0.0085  Temp3: -0.00001, 0.00007  y-intercept: -6.1024, -5.3836 | F1, 84 = 0.48,  P = 0.489 |
| *Co* | R2 = 0.98, R2adj = 0.97, F2, 3 = 241.6, p < 0.001;  µ4 =exp(-5.822 – 0.1749 × Temp + 0.0070 × Temp2 + 0.00002 × Temp3 | Temp: -0.1937 – -0.1573  Temp2: 0.0053 – 0.0084  Temp3: -0.00001, 0.00007  y-intercept: -6.1462, -5.4978 |
| *µ3* vs*µ4*for each species | *Oo* |  |  | F1, 270 = 1.86,  P = 0.173 |
| *Co* | F1, 270 = 6.26,  P = 0.013 |

**References**

Avramenko, R.W., Redman, E.M., Roy, L., Murilo, A.B., Palmeira, B.M., Yazwinski, T.A., and Gilleard, J.S., 2017. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. Int. J. Parasitol. 47, 893–902.

Avramenko, R.W., Elizabeth M.R, Roy, L., Yazwinski, T.A., Wasmuth, J.D., and Gilleard, J.S., 2015. Exploring the gastrointestinal ‘nemabiome’: deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS ONE 10, 1–18. https://doi.org/10.1371/journal.pone.0143559.

Charlier, J., Höglund, J., Morgan, E.R., Geldhof, P., Vercruysse, J., Claerebout, E., 2020. Biology and epidemiology of gastrointestinal nematodes in cattle. Vet. Clin. North Am. Food Anim 36, 1–15.

Edmonds, M.D., Johnson, E.G., Edmonds, J.D., 2010. Anthelmintic resistance of *Ostertagia ostertagi* and *Cooperia oncophora* to macrocyclic lactones in cattle from the western United States. Vet. Parasitol 170, 224-229.

Geurden, T., Chartier, C., Fanke, J., Frangipane, A., Traversa, D., Von Samson-himmelstjerna, G., Demeler, J., Bindu, H., Bartram, D.J., Denwood, M.J., 2015. Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in europe. Int. J. Parasitol. Drug. 5, 163–71.

Grenfell, B.T., Smith, G., Anderson, R.M., 1986. Maximum-likelihood estimates of the mortality and migration rates of the infective larvae of *Ostertagia ostertagi* and *Cooperia Oncophora*, Parasitol. 92, 643–52.

Grønvold, J., Høghschmidt, K., 1989. Factors influencing rain splash dispersal of infective larvae of *Ostertagia ostertagi* (trichostrongylidae) from cow pats ot the surroundings. Vet. Parasitol. 31, 57–70.

Hildreth, M.B., McKenzie, J.B., 2020. Epidemiology and control of gastrointestinal nematodes of cattle in northern climates. Vet Clin Food Anim*.* 36, 59-71.

Jasmer, D.P., Wescott, R.B., Crane, J.W., 1987. Survival of third-stage larvae of Washington isolates of Haemonchus contortus and Ostertagia circumcincta exposed to cold temperatures. Proc. Helminthol. Soc. Wash. 54, 48-52.

Kaplan, R.M., 2020. Biology, Epidemiology, Diagnosis, and Management of Anthelmintic Resistance in Gastrointestinal Nematodes of Livestock. Vet Clin North Am Food Anim Pract*.* 36, 17-30.

Molnár, P,K., Kutz, S.J., Hoar, B.M., Dobson, A.P., 2013. Metabolic approaches to understanding climate change impacts on seasonal host–macroparasite dynamics. Ecol. Lett.16, 9-21.

Navarre, C., 2020. Epidemiology and control of gastrointestinal nematodes of cattle in southern climates. Vet Clin Food Anim*.* 36, 45-57.

Morgan, E.R., 2013. Detail and the devil of on-farm parasite control under climate change. Anim Health Res Rev. 14, 138–42.

Pandey, V.S., 1972. Effect of temperature on survival of the free-living stages of *Ostertagia ostertagi*. J. Parasitol. Res. 58, 1042–46.

Persson, L., 1974. The survival of eggs and infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* in solid cattle manure and urine. Zentralbl. Veterinarmed. B. 21, 677–91.

R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Rose, H., Hoar, B., Kutz, S.J., Morgan, E.R., 2014. Exploiting parallels between live- stock and wildlife: predicting the impact of climate change on gastrointestinal nematodes in ruminants. Int. J. Parasitol. Parasites Wildl*.* 3, 209-219.

Rose, H., Caminade, C., Bolajoko, M.B., Phelan, P., van Dijk, J., Baylis M., Williams, D., Morgan, E.R., 2016. Climate-driven changes to the spatio-temporal distribution of the parasitic nematode, *Haemonchus contortus*, in sheep in europe. Glob. Change Biol. 22, 1271–85.

Rose, H., Wang, T., van Dijk, J., Morgan, E.R., 2015. GLOWORM-FL: a simulation model of the effects of climate and climate change on the free-living stages of gastro-intestinal nematode parasites of ruminants. Ecol Modell. 297, 232–45.

Sauermann, C.W., Leathwick, D.M., 2018. Veterinary parasitology a climate-driven model for the dynamics of the free-living stages of *Cooperia oncophora*. Vet. Parasitol. 255, 83–90.

Smith, G., 1990. The population biology of the free-living phase of *Haemonchus contortus*, Parasitol. 101, 309-16

Sutherland, I.A., Leathwick, D.M., 2011. Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends Parasitol*.* 27, 176-181.

van Dijk, J., Sargison, N.D.D., Kenyon, F., Skuce, P.J.J., 2010. Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. Animal 4, 377–92.

Verschave, S.H., Charlier, J., Rose, H., Claerebout, E., and Morgan, E.R., 2016. Cattle and nematodes under global change: transmission models as an ally. Trends. Parasitol. 32, 724–38.

Vineer, H.R., 2020. What modeling parasites, transmission, and resistance can teach us. Vet. Clin. North Am. Food Anim. 36, 145–58.

Wang, T., van Wyk, J.A., Morrison A., Morgan E.R., 2014. Moisture requirements for the migration of *Haemonchus contortus* third stage larvae out of faeces. Vet. Parasitol. 204, 258-64.

Wang, T., Rose, H., Morrison, A., van Wyk, J., Bolajoko, M.B., Bartley, D.J., Morgan, E.R., 2018. Microclimate has a greater influence than macroclimate on the availability of infective *Haemonchus contortus* larvae on herbage in a warmed temperate environment. Agric Ecosyst Environ. 265, 31–36.

Wang, T., Avramenko, R.W., Redman, E.M., Wit, J., Gilleard, J.S., Colwell, D.D., 2020. High levels of third-stage larvae (L3) overwinter survival for multiple cattle gastrointestinal nematode species on western Canadian pastures as revealed by ITS2 rDNA metabarcoding. Parasites & Vectors. 13, 1–11.

Wang, T., Redman, E.M., Morosetti, A., Chen, R., Kulle, S., Morden, N., Mcfarland, C., Vineer H.R., Colwell, D.D., Morgan, E.R., Gilleard, J.S., 2021. Seasonal epidemiology of gastrointestinal nematodes of cattle in the northern continental climate zone of western Canada as revealed by internal transcribed spacer ‑ 2 ribosomal DNA nemabiome barcoding. Parasites & Vectors. 14, 1–13.