4.1. Final publishable summary report

4.1.1. Executive summary.

Infection with helminth parasites (roundworms and fluke) represents a significant economic and animal health and welfare burden on the European ruminant livestock industry. The sustainable control of these parasites is being threatened by global changes, including climate change, the emergence of drug resistance, environmental change and changes to European livestock farming systems. The overall aim of the GLOWORM project was, therefore, to mitigate against such global changes through improved monitoring, mapping and modeling of helminth infections and associated disease in ruminant livestock.

Firstly, the economic burden of helminths on ruminant production in Europe was established from the available scientific literature, providing a baseline against which future threats and mitigation can be assessed. In order to monitor current infection levels in livestock, a panel of novel multi-parasite diagnostic tests has been developed. These tests can be applied to blood, milk and faecal samples to simultaneously detect and quantify key helminth infections at the individual animal or herd/flock level and have significant advantages over existing methods in terms of cost, speed and throughput of samples. Novel DNA-based tests have been developed and applied to survey the anthelmintic resistance status of the most important cattle and sheep gastrointestinal nematode (GIN) species in key European regions. To complement these tests, updated and practical methods for faecal egg counting (FEC) have been developed for quantifying GIN and fluke burden and an online calculator has been developed for assessing anthelmintic treatment efficacy through the faecal egg count reduction test (FECRT). In order to monitor parasitic helminth abundance, seasonality and geographic distribution, cost-efficient spatial sampling strategies and modelling approaches were developed. These methods were implemented in Europe on a large scale for dairy cattle on a West-East transect covering Ireland, Belgium, Sweden, Germany and Poland. As a result, the first pan-European map depicting the risk of occurrence of *Fasciola hepatica* in dairy cattle, based on bulk milk tank samples and a large dataset of remote sensing and ground-measured environmental data, was produced. For sheep, systematic data were collected on a North-South transect including Ireland, Switzerland and Italy, yielding improved geo-statistical techniques for spatial mapping. Based on obtained results, spatial sampling guidelines and planning tools were developed and made available for wider use by researchers and the livestock industry. In order to forecast the risk of infection and the implications of global changes, parasite transmission models have been established for the most important helminth species. Simulations focused on the effect of climate and other environmental changes on patterns of infection and explored possible mitigation strategies, including the management of anthelmintic resistance. In this context, targeted treatment (TT) and targeted selective treatments (TST) were applied under a range of farming conditions and found to reduce anthelmintic use whilst maintaining animal performance. TT and TST strategies can offer economic benefits compared to traditional, routine frequent anthelmintic treatment approaches. The level of impact is determined by several, easy-to-measure factors, including herd size and infection level. A framework for the incorporation of decision support systems for anthelmintic use has been developed, and several online tools have been developed, including software to measure the effect of worm infection on milk production and the economic effects of various treatment decisions. Finally, dissemination of project results was considered to be vital and was fulfilled through a series of different dissemination activities. These included 55 scientific peer-reviewed publications, 148 conference and poster presentations and 70 farmer and other stakeholder meeting events, during the lifetime of the project, ensuring a valuable GLOWORM legacy.
4.1.2. Description of project context and objectives.

Over the course of 3 years members of the GLOWORM consortium have worked to develop and optimise:

1. High-throughput diagnostic/detection methods and health indicator tools for monitoring ruminant helminth infections, including anthelmintic resistance
2. Spatially and temporally explicit predictive models of parasite abundance, which incorporate farm management as well as climatic and other environmental factors
3. Sustainable helminth control strategies to maintain animal productivity and drug efficacy

The concepts underpinning the GLOWORM project have emerged from the extensive research experience of the consortium, the findings from recent EU-funded livestock helminth research (PARASOL and DeLIVER) and on the EU’s consideration of the impact of parasitoses upon ruminant health, welfare and productivity (DISCONTOOLS).

Helminth parasitoses have a significant impact on the sustainability and efficiency of livestock farming in Europe

It is inevitable that the production of meat and dairy products will also have to expand to meet the demands of an exponentially increasing world population. Efficient ruminant livestock production is key to achieving this goal. In this context the global competitiveness of European livestock farming will largely depend on the degree to which this industry can achieve sustainable optimal production levels under changing environmental and socio-economic pressures.

All grazing animals are exposed to helminth infections at pasture and any respective future intensification of livestock farming will increase the risk of helminth disease. Gastrointestinal nematodes (GIN) and liver fluke have been ranked as the top two causes of lost productivity in ruminants by the recent DISCONTOOLS programme. The major economic impact of parasitism is due to sub-clinical infections causing production losses that affect the production of meat and milk and the value of fibre, fleece/hide and skin products. These costs have become increasingly important in the current economic climate with the low profit margins from the livestock sector. At present we only have a limited and fragmented understanding of the true costs of helminth parasitism including associated costs for its control in the EU.

Worldwide, ruminant livestock farming accounts for 70% of agricultural land use, occupies 30% of the ice-free surface of the planet and produces some 40% of the global agricultural Gross Domestic Product (GDP). Set against this, it is estimated to account for up to 18% of worldwide greenhouse gas emissions (Steinfeld et al., 2006). There is a new requirement for European livestock farming to become carbon emission efficiency driven (Gill et al., 2010). Animals emitting greenhouse gasses while not growing or producing because of illness add significantly to a farm’s environmental footprint (Gill et al., 2010), so combating infectious and parasitic diseases on livestock farms is essential for any improvements in the efficiency of production (Thornton, 2010).

Increased risk of helminth infection in livestock due to global change

The changing environment

In recent years, sharp increases in helminth-associated disease frequency and intensity have been reported within the European ruminant sector (van Dijk et al., 2010). Climate change, which increases the developmental success of parasites, may be one driver behind this trend. For example, there have already been reports of altered seasonal patterns of nematode and liver fluke infections in northern parts of the UK (Sargison et al., 2007; Kenyon et al., 2009). In Switzerland unpublished data (Hertzberg et al., unpublished) suggests *H. contortus* transmission is occurring at higher altitudes than previously recorded and in Sweden transmission occurs near the Arctic Circle (Lindqvist et al., 2001). If these trends continue as predicted, European farmers may be faced with new and unfamiliar parasitological challenges they are ill-equipped to meet. However, the study of
the effects of climate change on endemic diseases of livestock is still in its infancy (de la Rocque et al., 2008; van Dijk et al., 2010), and the effects of additional factors, such as altered land use, the emergence of anthelmintic resistance and farm management practices, have received little detailed attention (Morgan and Wall, 2009). GLOWORM aims to incorporate these additional factors in any prediction of altered infection risk due to climatic change.

**The importance of anthelmintic resistance (AR)**

An equally important driver of global change is likely to be treatment failure, which is being reported ever more frequently. The increasing occurrence of AR in worm populations threatens the sustainability as well as the efficiency of European livestock production. Although there are a number of different approaches to the control of helminth parasitism in livestock, at present efficient control relies almost exclusively on effective anthelmintic drugs. Most of these compounds have broad-spectrum activity i.e. they kill all the common roundworm species (there are specific products effective against fluke), but effective control ultimately relies on multiple treatments each year, particularly in young grazing stock. When first introduced, all these drugs were highly efficacious, but frequent and widespread use has resulted in the emergence of resistant parasite populations such that AR is now a major global problem, especially (but not exclusively) in small ruminants (Kaplan, 2004) and is the greatest threat to the sustainable control of helminthoses in the short to medium term. Anthelmintic inefficacy and resistance has also become apparent in cattle, reported initially in the Southern Hemisphere (Familton et al., 2001; Sangster and Dobson, 2002, Sutherland and Leathwick, 2011) but more recently within the EU (Demeler et al., 2009; El-Abdellati et al., 2010).

There is an urgent need to minimise anthelmintic usage e.g. by timing treatments optimally, taking into account climate change-driven alterations in parasite seasonality. To maintain or, in some regions, regain control over these parasites, an improved understanding, and quantification of the key mechanisms involved in their spatial and temporal prevalence is also paramount.

**Objectives**

GLOWORM aimed to maintain the success of European livestock production, by employing a multi-disciplinary approach to deliver new strategies and recommendations for monitoring and surveillance as well as technical tools for the sustainable control of helminth infections in grazing livestock. The development and implementation of innovative, refined approaches to worm control, targeted at the appropriate regional scale, is a prerequisite for reducing the enormous burden helminth parasitism imposes upon ruminant livestock production. This goal was considered to be best achieved by exploiting developments in high-throughput technologies coupled to novel diagnostics to identify sub-clinical infections within an affordable and flexible diagnostic capacity (Thornton, 2010). However, the provision of innovative diagnostic tools to identify the species implicated in infections, and individual and/or herd/flock markers of helminthoses, are only the first steps in the process. Any new diagnostic capacity needs to integrate with predictive disease risk forecasting models and newly developed livestock management software. Statistically-based risk models of helminthoses that incorporate Geographical Information System (GIS)-based surveillance, as well as temporally explicit models predicting periods of high risk, will inform farm-management responses to regional environmental, climatological, parasitological and socio-economic changes.

**Scientific and technical objectives of GLOWORM**

The main scientific objective of the GLOWORM project was the development of effective, sustainable strategies for treatment and control of ruminant helminthoses in the face of global change. This was achieved by developing innovative technologies that can be used to monitor and predict changing patterns of infection and disease for optimal anthelmintic usage, thereby limiting the development and spread of drug resistance.
Reaching these objectives required new scientific information. Our *scientific objectives* were to provide means of optimising:

1. Diagnosis of helminthoses in European livestock at the individual and herd level with specific attention to multi-species infections and the distribution of anthelmintic resistance in European livestock.
2. Prediction of the impact of global changes on the epidemiology of parasitic infections as well as the distribution and spread of anthelmintic resistance in European livestock.
4. Strategies for the sustainable management of helminth infections in a changing European agricultural landscape

In order to achieve the scientific objective of sustainable parasite control, our *technical objectives* were to:

1. Provide high-throughput diagnostic/detection methods for monitoring ruminant helminth infections and anthelmintic resistance
2. Develop epidemiological and economic tools, which take into account the impacts of global change on helminth disease risk and its economic consequences
3. Improve disease surveillance and forecasting systems based on the establishment of risk maps for the economically important helminth species affecting key European ruminant industry regions
4. Develop optimised anthelmintic resistance monitoring and treatment strategies in order to establish guidelines for sustainable helminth control.

Inter-relationships between work packages, proposal objectives and stakeholders are demonstrated in Fig. 4.1.2.1.
References:


4.1.3. A description of the main S&T results/foregrounds

Table 4.1.3.1 List of Participants

<table>
<thead>
<tr>
<th>Part. Nr</th>
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<tr>
<td>1</td>
<td>Jozef Vercruysse, Johannes Charlier - Ghent University Faculty of Veterinary Medicine, Merelbeke</td>
<td>UGENT</td>
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<td>2</td>
<td>Guy Hendrickx, Els Ducheine - Avia-GIS, Zoersel</td>
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<td>8</td>
<td>Fiona Kenyon, Philip Skuce - Moredun Research Institute, Edinburgh</td>
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<td>10</td>
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<td>14</td>
<td>Paul Torgerson (Section of Epidemiology), Hubertus Hertzberg (Institute of Parasitology) - Vetsuisse Faculty University of Zürich, Zurich</td>
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4.1.3.1. WP 1 - Development and field application of innovative diagnostic tools

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<tr>
<td>Work package Leader</td>
<td>Janina Demeler, Vice-leader: Philip Skuce</td>
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<td>Participant number</td>
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Background

Most, if not all, of the currently available diagnostic tests to identify helminth infections in livestock and to detect anthelmintic resistance (AR), have their limitations. These tests are typically labour-intensive, time-consuming and expensive, thereby limiting their application in veterinary diagnostic laboratories, field-testing on-farm or for large-scale surveillance. Another complicating factor is that grazing ruminants are typically infected with multiple helminth species at the same time, with some species being more pathogenic than others e.g. liver fluke or being predisposed to survive anthelmintic treatment e.g. *Haemonchus contortus*. The use of high-throughput immunological and DNA-based technologies offers the potential for rapid multi-species diagnostic applications, thus reducing costs and enabling diagnosis at a scale suitable for large-scale monitoring purposes. These technical advances in diagnostic capability will be complimented by optimization and

3 For the funding scheme: Research for the benefit of Specific Groups: SMEP, RTD Performer, SME Association, Other
standardization of faecal egg counting methods for detection of helminth infections and for identification of AR in worms and fluke in the field.

Objectives
To develop rapid, high-throughput diagnostic tests for the detection and quantification of the key gastrointestinal nematodes (GIN) and fluke in cattle and sheep. Specifically: (i) to develop an immunologically-based multiplex test for the simultaneous detection and quantification of *Cooperia oncophora*, *Dictyocaulus viviparus* (lungworm) and *Fasciola hepatica* (liver fluke) in cattle serum/milk samples; (ii) to develop a DNA-based multiplex diagnostic test for the detection and quantification of the major GIN species infecting cattle and sheep based on faecal samples; (iii) to develop a rapid test for the presence of *Haemonchus contortus* in sheep faecal samples (iv) to survey the benzimidazole resistance (BZ-R) status of the most important GIN species infecting cattle and sheep in key European regions by molecular genotyping; (v) to adapt the standard faecal egg count (FEC) for large-scale surveys and monitoring purposes and (vi) to evaluate and standardise the faecal egg count reduction test (FECRT) for field testing of anthelmintic efficacy in GIN and fluke.

Materials and methods
Both the immunological and DNA-based multiplex tests were based on the versatile Luminex® diagnostic platform. **Luminex® triplex immunoassay for cattle serum/milk samples:** For immunological test development, recombinantly-derived antigens were generated for each of the three cattle helminth species under test, namely, *C. oncophora* (14.2KDa ES antigen), *D. viviparus* (major sperm protein) and *F. hepatica* (cathepsinL). Each antigen was covalently coupled to carboxylated magnetic beads and tested in 3-5 different concentrations to determine optimal concentrations. Standard curves using a wide range of positive serum samples for the respective parasite species were established and cross-reactivity assessed. All samples (serum and milk) were pre-tested using established ELISAs for the detection of *D. viviparus* and *F. hepatica* and results compared to those obtained with the new Luminex® assays.

**Luminex® GIN species identification assay based on faecal samples:** For rapid and sensitive species differentiation of cattle/sheep GINs, a DNA-based multiplex assay was developed on the Luminex® platform. As a prerequisite for this, a simplified protocol for extracting DNA from eggs derived from faecal samples was developed [1]. Initially, probe design was conducted using the 28S rRNA, a target region known for its low genetic variation within different isolates of the same species. Specificity of probes was tested in a Reverse Line Blot hybridization assay. Optimal conditions for probe-sample binding were determined and modifications made where necessary to the stringency washing procedure to enhance probe specificity. Plasmid DNA of the 28S rRNA of nine different GIN species was used as template for assay validation. *Haemonchus* LAMP assay: A prototype LAMP assay has been developed, targeting the ITS-2 rRNA gene, for the sensitive detection of the highly pathogenic GIN, *H. contortus* [2]. The method was evaluated for sensitivity and specificity and compared with routine PNA lectin staining of *Haemonchus* eggs and the equivalent real-time PCR protocol.

**Benzimidazole resistance (BZ-R) survey in cattle and sheep:** Pyrosequencing assays for the detection of BZ resistance-associated SNPs in the β-tubulin of the cattle parasites, *C. oncophora* and *O. ostertagi* were developed and successfully applied to both species. Samples were derived from experimentally infected cattle and field samples from South America, Europe and Australia. SNPs at all three positions (codons 167, 198 and 200) were detected and quantification of BZ-R status, as determined by allele frequency, was possible. The newly developed assays have been used for a small-scale BZ resistance survey on cattle farms in Belgium, Ireland and Germany in 2013. For sheep nematodes, species-specific pyrosequencing assays have been developed for the key GIN species, namely *H. contortus*, *T. circumcincta* and *Trichostrongylus* sp., the latter assay being
developed specifically for this purpose [3]. Field samples (faeces) from sheep were collected as part of the spatial sampling strategy on ovine farms in Ireland, Italy and Switzerland (WP3.1). For each farm, faecal samples were analysed by FLOTAC for FEC and extracted eggs coprocultured to the L3 stage. A sub-sample was subsequently identified morphologically to species/genus level for reference and the remainder archived as a biobank in ethanol for molecular genotyping studies. Representative samples were collected in both 2012 and 2013 and from the same farms where possible.

Adapting FEC for large-scale monitoring: Existing knowledge of the error structure of FEC, including pooled FEC, was used to generate a statistical framework for FEC observations, based on well-grounded assumptions of parasite over-dispersion between individual animals within a herd or flock, and Poisson error in egg counts [4]. This framework was applied to predict FEC and associated inaccuracies, under various combinations of variables. Outcomes were predictions of how FEC sample design affects performance, and, equivalently, optimised protocols for different aims and demands. The viability of pooled FEC as a reflection of underlying mean group FEC was challenged experimentally by comparing pooled and mean individual FEC across a range of groups of sheep, goats and cattle in the different participating countries. This included evaluation of the novel pen-side FEC format, mini-FLOTAC, alongside existing established tests [5, 6].

FECRT for field-testing of anthelmintic efficacy in GIN and fluke: The statistical framework above was extended to examine the performance of different FEC regimes in detecting poor anthelmintic efficacy. A number of in silico trials were undertaken, in sheep and cattle, using pooled and individual FEC, and using different FEC methodologies. Pooled FECs were applied alongside individual FEC to detect anthelmintic resistance in the field, to determine whether pooled FEC could contribute to more affordable monitoring of drug efficacy on farms in Europe.

Results

Luminex® triplex immunoassay for cattle serum/milk samples: Compared to existing ELISA tests, greater sensitivity was observed for both serum and milk Luminex® immunoassays. Especially for *F. hepatica*, agreement between milk and serum samples was improved over the existing Svanovir® ELISA, particularly since the ELISA detected a considerable number of milk samples as false positive while the corresponding serum samples remained negative. The new test proved to be more sensitive than existing ELISA tests for both lungworm and liver fluke and has been evaluated on serum and bulk tank milk samples from naturally infected livestock in key European regions (Belgium, Poland, Germany, Ireland, n= >4000), collected in WP3 (e.g. Fig.4.1.3.1.1). Results indicate the prevalence of lungworm to be relatively low across all 4 countries (range, 0-5.8%), that liver fluke is highly prevalent in Ireland (93.6%) and that *Cooperia* is relatively common across all European regions surveyed (range, 31.7-73.1%). The new test offers advantages over existing tests in terms of speed and throughput and being able to simultaneously test for 3 key species using the same sample set.

![Figure 4.1.3.1.1 Example Q-GIS map for Germany after examination of milk samples with Luminex® triplex assay.](image-url)

...an unambiguous species...
possible using the newly developed Luminex® method. Due to extreme sequence conservation between the three trichostrongylid species (viz. *T. axei*, *T. vitrinus*, *T. colubriformis*) a pan-trichostrongylid-probe was designed, which discriminates these species from others at genus level. This was also performed for *Ostertagia ostertagi* and *Teladorsagia circumcincta*, as the sequences of these two species were, with one exception, identical. As these two species are found in different hosts (cattle and small ruminants, respectively), this should have no implications for the relevance of this assay in practice. The entire procedure can be performed within 3 to 5 h depending on the number of samples processed.

**Haemonchus LAMP assay:** A prototype LAMP assay has been developed for the sensitive detection of the highly pathogenic GIN, *H. contortus* [2]. This method is rapid (<1h), requires little/no DNA extraction or sophisticated equipment and results can be visualised by eye. The assay can detect *H. contortus* DNA in a faecal sample containing 1 egg per gram of faeces and does not cross-react with other common GIN or fluke species. Fluorescence detection on a real-time PCR platform demonstrates that the LAMP assay is semi-quantitative. Recent developments in LAMP chemistry and assay design have significantly improved this aspect of the LAMP assay and are currently being evaluated using samples collected by P9 and material from IT, ROI and CH, collected as part of Task 1.3.

**Benzimidazole resistance survey in cattle and sheep:** The results from the BZ-R survey are shown in Fig. 4.1.3.1.2. Following key findings were made: (i) for cattle, BZ resistant allele frequencies were relatively low (max. 13%) in the countries sampled, with codons 167 and 200 being equally prevalent; (ii) for sheep, the SNP in codon 200 was the most common determinant of BZ-R across all 3 countries and in all 3 species, reaching allele frequencies of 90-100% in some cases e.g. *Haemonchus* & *Trichostrongylus* in Switzerland; the codon 200 SNP is also the dominant feature of BZ-R *T. circumcincta* in Ireland; (iii) in directly comparable farms, there has been an increase in codon 200 BZ-R allele frequency from 2012-2013, with a concomitant reduction in 167 & 198 e.g. *Teladorsagia* in Italy (iv) Switzerland had the highest BZ-R allele frequencies, followed by Ireland and Italy [3].

![Fig. 4.1.3.1.2 BZ resistance-associated SNP frequencies at β-tubulin codon 200 in major GIN species across key European regions in 2013.](image)

**Adapting FEC for large-scale monitoring:** Literature surveys collected realistic values for variables governing FEC accuracy, such as degree of FEC aggregation in groups of grazing animals: moment estimates of negative binomial parameter *k* varied widely but clustered between 0.5 and 2.0. The statistical modelling revealed that the number of animals included in pooled FEC had a dominant influence on their accurate estimation of the true underlying group mean FEC. A minimum of 10-15 individual samples is needed under normal circumstances, and more at higher levels of aggregation. Dilution factor was important under some circumstances (e.g. at low FEC, such as after anthelmintic treatment) but not in most monitoring situations. The mass and uniformity of faecal samples taken for pooling was not as important. Along with development of the mini-FLOTAC FEC method, results give confidence in the application of pooled FEC for monitoring of FEC on farms in support of targeted treatment. Protocols for representative FEC estimation using pooled FEC were produced and applied in the large scale cross-sectional surveys undertaken in WP3. Field data showed that pooled FEC provided highly representative estimates of group mean individual
FEC in sheep, goats and cattle.
FECRT for field testing of anthelmintic efficacy in GIN and fluke: FECRT design was explored using statistical modelling, and dilution factor shown to be of crucial importance, alongside the number of individuals sampled, in the accuracy of FECRT results in relation to known underlying *in silico* drug efficacy [7, 8] (Fig. 4.1.3.1.3). Through members of the consortium, these findings are being fed into updated international standards for the detection of drug resistance in helminths of veterinary importance. Pooled FEC were shown to be robust, in theory and in the field, as indicators of anthelmintic resistance, though interpretation could be difficult in cases of marginal resistance [9]. The statistical models were made publicly available as freely shared R software ‘eggCounts’ and also as a user-friendly online calculator (available at http://www.math.uzh.ch/as/?calc), which is already in widespread use among researchers and shows promise for further refinement for practical uptake by advisors [10]. Pooled FECRT were further evaluated for the liver fluke, *F. hepatica*, and found to be effective, with coproantigen measurement serving to reduce the time at which results are available [11].

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*Fig 4.1.3.1.3* The detection of reduced (true drug efficacy < 95%) and normal drug efficacy (true drug efficacy ≥ 95%). The white zone indicates which drug efficacies can be accurately determined by FECRT as having a reduced efficacy, whereas the black zone indicates which drug efficacies can be accurately determined as having a normal efficacy. The grey zone indicates drug efficacies that cannot be accurately determined as having either reduced or normal efficacy. Results show important interactions between variables, e.g. increased uncertainty when FEC are highly aggregated (low k) (compare lines 3 and 2), which can be offset by using methods with higher analytical sensitivity (lower detection limit (compare lines 2 and 1)).

**Conclusions**
WP1 has developed novel high-throughput and species-specific diagnostic tests to detect GIN and fluke infections in ruminant livestock and to determine anthelmintic resistance status of the most important species. These have been extensively validated using experimental and field-derived material and deployed to survey GIN and fluke in key European regions. These technological advances have been complimented by simplification and standardisation of routine faecal egg counting methods, based on robust statistical sampling and interpretation criteria for large-scale monitoring and surveillance purposes. Taken together, this panel of new and improved tests enhances our diagnostic capabilities for detecting and monitoring the impacts of global changes on helminth parasites in ruminant livestock in Europe and beyond.

**Practical outcomes**
A novel immunoassay has been developed, which offers researchers the ability to simultaneously detect antibodies produced against *C. oncophora*, *D. viviparus* and *F. hepatica* in bovine serum and milk samples. This assay is characterised by high sensitivity, specificity and reproducibility, and has obvious potential for high-throughput screening and automisation. The Luminex® platform has the
ability to include other herd health parameters, such as hormones and inflammatory biomarkers. A prototype DNA-based assay for GIN species identification and quantification of cattle and sheep GIN, based on faecal samples, has also been developed, using the same diagnostic platform. Furthermore, a LAMP assay has been developed for the sensitive and specific detection of H. contortus DNA in sheep faecal samples, which has obvious potential for pen-side applications. A panel of species-specific pyrosequencing assays has been developed for the major GIN species infecting cattle and sheep and deployed to survey benzimidazole resistance in key European regions. Sampling strategies and protocols for composite FEC have been devised for large-scale GIN and fluke monitoring, including assessment of drug efficacy, and an online calculator developed for robust interpretation of FECRT data.

Scientific output and references


4.1.3.2. WP 2 - Parasite transmission models

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Background

Effective intervention against parasitic disease is temporally sensitive, relying on the coincidence of animal grazing with infective stage availability. The timing of treatment and other actions, including grazing practice and diagnostic monitoring in support of targeted treatment strategies, is therefore very important to the outcome in terms of the reduction of disease challenge and selection for anthelmintic resistance. Moreover, one of the main effects of climate change on parasite epidemiology is likely to be the altered seasonal availability of infective stages. Therefore, comprehensive modelling approaches are needed that consider temporal patterns of infection, by building and populating mechanistic representations of the life-cycles of the major helminth species. Model components should explicitly incorporate climatic stochasticity, forage availability and utilisation, and variation in farm management at the regional level. Outputs can be extended to global change scenarios through variation in climate, farm systems and parasite biology, as well as,
potentially, parasite adaptation. The extent to which parasite adaptation to changes in climate and management will affect their control has barely been considered in trichostrongyles beyond drug resistance. Explicit prediction of the timing of helminth transmission by realistic models is needed to optimise control strategies. Such models could be applied on farms as decision support tools, and to horizon-scan for disease threats by industry and animal health authorities and to guide policy at national and supra-national levels.

**Objectives**

To assemble, run and validate process-based transmission models of the major helminth species, supporting future control strategies for Europe under climate change scenarios. Specifically: (i) Estimate key parameters for transmission and economic models, using systematic review and experimental studies of the effects of climate on environmental stages; (ii) Refine the models by taking into account projected changes in grass growth and farm management patterns in Europe, and parasite adaptation through trait evolution; (iii) Construct temporal models of parasite transmission that use these data to predict the seasonality and pressure of infection in grazing livestock; (iv) Investigate strategic control options under climate change scenarios, linking to WP4; (v) Validate predictions.

**Materials and methods**

Model frameworks were developed by extending previous attempts, incorporating refined representations of the ecology of the free-living parasite stages, capacity to include climatic stochasticity and climate change scenarios. Parameters were estimated from the literature and unpublished data, and knowledge gaps used with model sensitivity analysis to target further experiments. A series of experiments were conducted to provide missing parameters for the models. Farm management data such as grazing season length were compiled from existing databases and new questionnaire surveys, and used to add realism to model exploration of current and future parasite epidemiology. The capacity of the target parasites to adapt to climate change was assessed by laboratory comparison of key climate-related performance indicators between populations in northern and southern Europe. Model outputs were validated using previously available and new longitudinal field data, and used to explore changing patterns of infection under global change scenarios, along with potential mitigation measures. The economic impact of helminths in ruminants in Europe was estimated by systematic review and meta-analysis.

**Results**

Model frameworks were developed to predict parasite transmission patterns under current and future climate and management. Process-based transmission models that predict temporal (seasonal and between-year) variation in levels of infection are needed to provide useful support for parasite control strategies. The over-arching objective of WP2 was to develop models to predict parasite challenge and transmission patterns at regional level in Europe, given assumptions of current and future grazing management and parasite control. These can eventually be used to provide decision support for parasite control under various scenarios. Model frameworks were developed for the target nematode species. Two generalised, complementary model frameworks were developed to simulate the population dynamics and transmission of gastrointestinal nematodes in sheep and cattle (Fig. 4.1.3.2.1). The models were adapted for the key target species *H. contortus* and *T. circumcincta* in sheep and *O. ostertagi* and *C. oncophora* in cattle. Predictions were validated using data from the literature and new field data collected during the project.
Simulations using the GLOWORM-FL model and current and future climate predicted an overall increase in the development and survival of *H. contortus* and *T. circumcincta* on pasture in a temperate region, but an overall decrease in the development and survival of *O. ostertagi* on pasture. There was an interaction between increased temperature-dependent development and mortality rates and moisture limitations during the summer months which resulted in a net decrease in populations of nematodes on pasture [1].

Validation of models against independently collected data is a vital component in their development. The GLOWORM-FL framework was validated thoroughly for temperate regions using data from the literature and new longitudinal data. Comparison of GLOWORM-FL output with the output of a previously published model and repeat observations of L3 availability on pasture between November and April in the UK showed that GLOWORM-FL improved significantly on previous models by incorporating the migratory behaviour of the nematodes on pasture. Simulations using the GLOWORM-FL model and an instantaneous adaptation of the GLOWORM-PARA framework with and without regionally variable management were run for *O. ostertagi* in adult dairy cattle in Germany, Belgium, Ireland and the UK. The predicted magnitude of exposure to infective larvae was compared with the antibody levels measured in bulk-milk samples during the EU FP6 PARASOL project, which are also representative of exposure. There was no significant association between measured antibody levels and AUC for simulations using only climate, but there was a positive association between ODR and AUC when management was included. The models were also validated against new data from plot trials in the Swiss Alps, in which an elevation gradient acted as a surrogate of climate warming. Plots were seeded with faeces containing *H. contortus* eggs and larvae periodically recovered from herbage. Infective larvae were recovered at 2,000m altitude, even though this was predicted not to occur based on local air temperature. This result suggests that the microclimate at the soil surface could be important to larval development and should be measured for local application of transmission models.

The model frameworks provide tools to explore the impact of management strategies on parasite populations and subsequent infection pressure. Simulations using the GLOWORM-FL framework were used to assess the likely impact of climate and helmintth treatment strategies on the population of drug-susceptible nematodes on pasture (refugia) at the time of spring turnout of lambs, and therefore qualitatively assess the risk of development of anthelmintic resistance. Results differ markedly when using different climate scenarios and are not presented in detail here. However, the models can be easily adapted to different climatic and management assumptions, and will provide a strong basis for assessing likely effects of different intervention strategies on infection patterns and the effectiveness of refugia generated by TT and TST strategies.

A complementary ‘Q<sub>0</sub>’ modelling approach was devised for gastrointestinal nematodes. Q<sub>0</sub> (basic reproduction quotient) is a simple model that captures the suitability of instantaneous climatic conditions for population growth. Although more limited than the frameworks described above to fully integrate management complexity, this method can capture trade-offs in development and death rates. Q<sub>0</sub> models were developed for *H. contortus* and applied on a spatial scale in WP3, and
for *C. oncophora* and *O. ostertagi*, to compare the relative predicted effects of global warming on transmission potential. Exploring a simple year-round warming scenario, for the UK, the model predicts that *C. oncophora* is likely to benefit more from global warming than *O. ostertagi*, though to a varying extent in different parts of the UK. It is further predicted that warming may have a net negative effect on *O. ostertagi* populations in the warmer temperate regions and altitudes, in agreement with the GLOWORM-FL model above. Both models can be extended to other European regions. The nematode model framework was adapted and extended to the more complex life cycle of *F. hepatica*. Example model output, tracking the seasonality of individual stages in the life cycle, is shown in Fig. 4.1.3.2.2. The model captures expected seasonality and can be refined and applied to management scenarios.

A bespoke model was devised to predict hatching of infective larvae of the nematode *Nematodirus battus* at pasture. The unusual biology of this parasite leads to peaks of infection in spring-born lambs with high morbidity and mortality, especially in NW Europe. A simple degree-day model was built using experimental data from the literature, and applied to provide a regional hatching date forecast for the UK. This was accessed by several thousand users in 2013 and 2014 and incorporated into stakeholder advice channels.

Systematic reviews and meta-analyses were conducted to estimate parameter values for the transmission models. Better understanding of the climate-dependence of helminth development, survival and translation onto herbage is needed to support transmission models that are accurate and useful in meeting the challenges of global change. Parasitic phase parameters were reviewed for the nematodes of cattle, *O. ostertagi* and *C. oncophora*. The main parameters determining parasite density during the parasitic phase were estimated (Table 4.1.3.2.1). A systematic review was performed covering studies from 1962 to 2007, in which helminth-naïve calves were artificially infected with *O. ostertagi* and/or *C. oncophora*. The database was further extended with results of unpublished trials [2].

| Table 4.1.3.2.1 Vital rates of parasitic phase of gastrointestinal nematodes of cattle (± S.E.) | *O. ostertagi* | *C. oncophora* |
| Establishment rate | 0.269 (± 0.022) | 0.286 (± 0.029) |
| Proportion of larvae entering hypobiosis | 0.041 (± 0.009) | 0.007 (± 0.002) |
| Daily mortality rate | 0.028 (± 0.002) | 0.039 (± 0.005) |
| Daily fecundity rate per female | 429 (± 68) | 2744 (± 815) |

Parameters for the free-living stages of keystone nematode species were estimated from the literature [1]. Effects of temperature on the development and survival of larvae of *H. contortus* and *O. ostertagi* were characterised and regression used to derive functions for the transmission models. Significant gaps in the literature were identified, especially for temperature effects in other species, and effects of moisture on larval availability. Sensitivity analysis of the transmission model was used to rank parameters based on their predicted effect on larval availability, taking into account...
ranges of uncertainty. In this way, parameters were prioritised for further experimental work. Parameters for the free-living stages of *Fasciola hepatica* were reviewed, and important gaps were similarly identified and prioritised for experimental study. These included survival of the free-living stages, development of and within snails, and effects of environmental variables other than temperature.

Experiments were conducted to improve knowledge of parameter values for entry into the models, and to advance understanding of the biology of the free-living stages of the target helminth species. Laboratory experiments focused especially on the effects of moisture and rainfall on the availability of infective nematode larvae and fluke metacercariae. A series of experiments was conducted in climate-controlled incubators and glasshouses to determine the moisture requirements for the migration of *T. circumcincta* and *H. contortus* between faeces and pasture [3]. These focussed on the effects of rainfall on larval emergence from faeces, a key gap in the literature and likely to be influenced by altered temperature-rainfall interactions under climate change scenarios. Key findings were the need for a threshold amount of rainfall to induce larval emergence, above which additional rainfall has little effect; an interaction between rainfall and faecal moisture content; and estimates of daily migration rates from faeces under optimal and suboptimal moisture conditions. The effects of temperature on the development and survival of *C. oncophora* were determined for the first time, filling another deficit in the published literature that seriously limited the predictive ability of the GLOWORM models. Mono-culture eggs and larvae were incubated at 14, 18, 22, 26 and 30 °C, and development success and development and death rates of larvae were determined. Results of these studies were combined with those of the systematic reviews to define the development, survival and behaviour of the free-living stages of *O. ostertagi*, *C. oncophora*, *T. circumcincta* and *H. contortus* [1]. A further series of experiments investigated the consequences of humidity for the availability of *C. oncophora* larvae, in a glasshouse and then a climate chamber [4]. Results revealed that larvae survived well for one week under drought stress, but not for 4-6 weeks; that plant mixtures with clover content increased parasite survival; and that larvae migrated into soil under drought stress, with soil therefore acting as refugium for larval survival. More larvae were recovered from soil than from herbage regardless of group allocation and treatment. In the controlled incubator experiment, humidity, temperature and illumination were regulated. Two scenarios were compared: a profile mimicking an average spring day in Germany, and a profile mimicking a relatively warm summer day in Germany. Grass pots containing *Lolium perenne* were prepared and L3 inoculated. Larvae were recovered from grass and soil, and the fitness of recovered larvae was evaluated by modified larval migration assay. Larval survival and fitness declined with time, while migration into the soil increased over time. Numbers of L3 recovered from soil were negatively affected by high temperature, but not by drought stress. Climate change in temperate regions will therefore likely affect L3 occurrence on pasture. Soil was identified as an important refugium for infective nematode larvae. Effects of temperature and moisture on *F. hepatica* eggs and meta-cercariae were also studied experimentally. Eggs survived well at temperatures as low as -6 °C, but died rapidly in colder conditions. In temperate oceanic climates, most eggs deposited during winter would survive until spring. Metacercarial survival was examined across a range of temperature and moisture. Temperature-dependent mortality rates were estimated and carried forward into the prediction models for *F. hepatica*. The frequency of water application to desiccated metacercariae did not significantly influence death rates, i.e. metacercariae were not able to rehydrate following desiccation.

The potential for adaptation of parasites to local climate and management conditions was assessed. Such adaptation could affect the accuracy of transmission model predictions, and govern the extent to which model predictions and changes in management and parasite control strategies are robust to parasite micro-evolution. Experiments were conducted to compare the climatic preferences of *F. hepatica* from different countries. It was hypothesised that populations from southern Europe (southern Italy) would be pre-adapted to show increased survival at higher temperatures and better
survival of desiccation, relative to those from northern Europe (Wales). However, the populations did not perform significantly differently at any temperature or simulated rainfall treatment, so these hypotheses were refuted. However, the shedding pattern of cercariae from Galba truncatula snails was very different when infected with fluke from these different regions. This may be due to adaptation to local snail populations/species or differences in optimum temperature for development of cercaria, or both. This interesting finding should be developed in future work. The temperature profile of H. contortus development and survival was also compared between populations. Work focussed primarily on observations of development success in field populations of H. contortus from Switzerland and Italy, and a laboratory passaged isolate of H. contortus (MHco3 originally derived from an East African isolate). Results suggest little difference between field isolates, conferring confidence in the transferability of the nematode models between European regions and to future climate change scenarios.

Data on farm management parameters in Europe were collected and compiled, to base the prediction models on realistic assumptions. The epidemiology of parasites of veterinary importance is driven not only by climate but also by farm management. Data on how grazing ruminants are managed in different regions of Europe are therefore essential to the performance of predictive transmission models and their relevance for parasite control. A questionnaire on farm management was devised and distributed using the GLOWORM network during the cross-sectional surveys on sheep farms conducted in Ireland, Italy and Switzerland within WP3. A database of typical farm management was also established. This utilised data from the farm accountancy data network (FADN), the EUROSTAT survey on agricultural production methods and an online questionnaire for farmers, administered through GLOWORM project participants and an independent network of researchers and farm advisors. Current grazing season length (GSL; months) on grazing dairy, beef and sheep farms across Europe was found to be highly correlated with bioclimatic variables in a multiple regression across 986 European NUTS 3 regions, being positively correlated with the mean temperature of the coldest quarter and isothermality, and negatively correlated with precipitation in the wettest month [5]. Results were used to map reported current mean GSL for dairy, beef and sheep farms in Europe. Extrapolating the original relationship to climate change scenarios predicted by CMIP5 suggested that GSL is likely to increase in most European regions, particularly in the north-east, due to increasing winter temperature (Fig. 4.1.3.2.3). However, variation in GSL between regions is also likely to increase and some regions may experience GSL shortening by up to 1.5 months. These results enabled estimates of turnout and housing dates, and future predictions for dairy, beef and sheep farms in 986 regions across Europe, to anchor the parasite transmission models. The annual proportion and amount of grazed grass in the diets of farmed ruminants was calculated for each of the EU 27 countries from net energy balances based on farm structural data live-weights, feed prices, forage tables and the INRA net energy system. Grass utilisation varied widely between countries and farm types and was least on dairy farms and greatest on sheep farms. An online survey was used to collect grazing management and parasite control data on dairy, beef and sheep farms in Ireland, the UK, Belgium and France. Most farmers predicted that the proportion of grazed grass in livestock diets would increase in future, potentially reflecting positive attitudes towards utilising more grazed grass as a feed source.

Figure 4.1.3.2.3 Maps of predicted future changes to grazing season length (GSL; months) on dairy farms under the CMIP5 HADGEM-ES climate change scenarios for 2050 (a, b) and 2070 (c, d) under representative concentration pathways 2.5 (a, c) and 8.5 (b, d). Maps were also produced for beef and sheep.
The economic impact of helminth infections was estimated at European scale. This is of central importance to the application of the prediction models at farm and regional levels, since optimal parasite control should be based on economic benefits. Although helminth infections are known to cause substantial economic losses, these have not previously been quantified in a systematic way. A systematic review was therefore conducted to determine the economic losses associated with nematode and *Fasciola* infections in Europe. Results provide a first analysis of the economic burden of gastrointestinal nematode and liver fluke infection to the EU livestock industry, suggesting total annual losses of €3-5Bn, including production loss and costs of control.

Conclusions

WP2 delivered a model framework that is well validated for the target parasite species, and sufficiently flexible to allow extension to different parasite species in future, and to a wider range of geographical locations and scenarios. The models are underpinned by a strong foundation of parameter estimates for parasitic and farm management components, which have been made freely available. Preliminary results on trait variation suggest that models are applicable to farming systems across Europe. Estimates of the economic impact of helminths in these systems at European level were made for the first time.

Practical outcomes

The models have great potential to assist decisions on sustainable and economically optimised parasite control at farm level. This was illustrated by the development of a model to predict hatching of *Nematodirus battus* larvae as a result of increasing temperature in spring. The model was made available as an online risk map and used by farmers and advisors in the UK in 2013 and 2014 to better target the timing of anthelmintic treatment. Further decision support tools are envisaged to arise from the GLOWORM model frameworks for other nematode species and liver fluke. The approach used in this project, especially the tight linkage of systematic review, model sensitivity analysis and experimentation, was also successful and will guide future scientific efforts towards more systematic use of modelling, to improve its practical application to problems of parasite control.

Scientific output and references


4.1.3.3. WP 3 - Multi-scale spatio-temporal modelling

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Background

The presentation of parasitological data based on interactive, computer-generated maps is a straightforward way of visualizing large numbers of datasets in a geographical context at various
Spatial scales (local, national, regional and global). Spatial risk models of helminth infections in ruminants that are based on Geographical Information System (GIS)-based surveillance inputs, as well as temporally explicit models predicting periods of high risk, are fundamental for farm-management responses to environmental, climatological and parasitological changes occurring in Europe. Representation of epidemiological data in the form of a map facilitates interpretation, synthesis and recognition of any changing frequency and pattern of helminth infections and the appearance of clusters of helminth distribution. Moreover, maps are convenient tools to foster discussion and dialogue among different stakeholders, the industry and animal health authorities in order to improve livestock farming in Europe. However, the use of GIS and other geospatial tools (e.g. remote sensing, global positioning systems, virtual globes, spatial decision supports, etc.) does by no means overcome the major concern of any research, namely data availability and data quality. Indeed, maps should be linked to diagnostics and further impetus for research on geospatial technology and links with standardized diagnostic techniques and robust models must take place. We emphasize the need for integrating sound epidemiological designs with innovative diagnostic tools and strategies and geospatial tools for mapping helminth infections of ruminants. Recognizing these challenges, standardization of sampling and laboratory procedures, and innovating, validating and applying new tools and strategies will foster and sustain long-term control of helminth infections of sheep and cattle in Europe.

Objectives
To provide practical responses to environmental, climatological and parasitological changes occurring in Europe. Specifically to: (i) Investigate cost-efficient and practical spatial sampling strategies; (ii) Construct a GLOWORM spatial database with parasitological, farm management, environmental and climatic data; (iii) Develop multi-scale spatiotemporal models for parasite occurrence, including the impact of climate change and anthelmintic resistance.

Materials and methods
Parasitological cross-sectional surveys were conducted on a representative number of sheep and cattle farms in selected pilot areas through Europe. The geo-referenced results were mapped to obtain an updated parasitological scenario of ruminant farms in Europe. A multi-scale GIS of parasitological, climatic, environmental and farm management data was constructed. A comparative analysis of parasite occurrence between the study areas distributed throughout Europe was conducted using different multi-scale spatial models, including Bayesian geostatistics, random forest trees and boosted regression trees, with and without environmental and climatic covariates. Furthermore the climate-based Ollerenshaw Mt model for *F. hepatica* was expanded and a Q0 model for *H. contortus* was developed, tested and validated over Europe. Existing data on the spatial distribution of anthelmintic resistance in ruminants in Europe were obtained by a systematic review of peer-reviewed literature.

Results
Standardized sampling procedures were developed for surveys of helminth infections in ruminants in Europe. A standardised set of cost-efficient and sustainable spatial sampling rules was designed and applied to acquire baseline parasitological data through cross-sectional surveys on the prevalence and distribution of helminths in sheep and cattle bred in the GLOWORM pilot areas located in: Ireland, Italy and Switzerland for sheep; and Belgium, Germany, Ireland, Poland and Sweden for cattle. Standardized sampling and diagnostic procedures (from the farm to the lab) were used (Fig. 4.1.3.3.1) in order to: (i) construct quality maps to be disseminated to practitioners, farmers, stakeholders and decision-makers and (ii) use quality data as input for the multi-scale spatial models [1].
The field surveys were conducted in 2012 and 2013 during the second half of the grazing season (August-October) to have a clear picture of the current distribution and prevalence of gastrointestinal nematodes (GIN), liver flukes and other parasites in sheep and *F. hepatica* in dairy cattle across Europe. Different sampling approaches (random sampling versus systematic sampling versus proportional allocation versus systematic grids versus preferential sampling) were compared in terms of cost-effectiveness and applicability on the field [2]. Standardized laboratory procedures were used for the analyses of the biological samples collected on sheep farms (pooled faecal egg counts by FLOTAC and coprocultures) and dairy cattle farms (standardized bulk-tank milk ELISA). All the farms sampled were geolocated taking into consideration legal privacy issues. With regard to dairy cattle, the geolocated parasitological results from 3,359 dairy cattle farms in selected regions from Belgium, Germany, Ireland, Poland and Sweden were used as input to construct models of occurrence at different spatial scales (country-based and pan-European models). Similarly, with regard to sheep, the geolocated parasitological results from 519 dairy sheep farms in selected regions from Ireland, Switzerland and Italy were used as input to construct models of occurrence at different spatial scales (local and country-wide models).

A multi-scale GIS of parasitological, climatic, environmental and farm management data was constructed. The GIS data layers (including metadata) covered the entire Europe and each country involved into the project, separately: Belgium, Germany, Ireland, Italy, Poland, Sweden, Switzerland, United Kingdom. The layers were made available to the different GLOWORM members via the web data archive. For each of the climatic and environmental variables (e.g. eco-climatic zonation, elevation, land cover, temperature, rainfall, soil types and hydrography), XML files were created, to be compliant with the INSPIRE rules.

Helminth distribution in Europe was analysed using different multi-scale spatial models. For both sheep and cattle, the geo-referenced results were mapped to obtain an updated parasitological scenario of ruminant farms in Europe. Prevalence results differed across the different EU countries, especially with regard to *F. hepatica* (high prevalence in Ireland and low prevalence in Switzerland and Italy) and *H. contortus* in sheep (low prevalence in Ireland; high prevalence in Switzerland and Italy). A comparative analysis of parasite occurrence between the study areas distributed throughout Europe was conducted using ecological analyses. Multi-scale spatial models were built combining Bayesian and other statistical approaches (e.g. random forest, boosted regression trees using VECMAP™), and using parasitological data collected during the field surveys as well as the compiled extensive set of climatic, environmental and socio-economic data (GIS datalayers) as an input. With regard to sheep, exploiting data derived from a preliminary first stage survey conducted on a regular grid in southern Italy, Bayesian geostatistical models were developed to predict the probability of infections by *F. hepatica* (Fig. 4.1.3.3.2), *H. contortus* and other parasites; prediction standard errors were related with each potential farm location [2]. These farm predicted risk probabilities and related uncertainty can be used for planning future surveys (second-wave surveys) as well as for optimization of intervention strategies.
With regard to cattle, multi-scale spatial modelling of *F. hepatica* occurrence in dairy cattle were created using the VECMAP™ software, at country-based level (Ireland, Germany and Poland) and at pan-EU level. Results of the Pan-European model of *F. hepatica* are given in Fig. 4.1.3.3.3.

This is the first Pan-European model of *F. hepatica* occurrence in dairy cattle. It was validated against the GLOWORM data and published national *Fasciola* risk maps. The results indicate that both Random Forests and Boosted Regression Trees (BRT) are capable of modelling the probability of exposure to the liver fluke; still the BRT model is better at discriminating between the low probability and the high probability infection areas [3] and may therefore be of better practical use.

The most important factors in both models are temperature and rainfall, as expected from the literature. The various outcomes from the different statistical approaches suggested that specific weather and environmental risk factors support robust and precise distribution models of helminths of sheep and cattle in Europe at country-based and EU level. The findings identified models with the ability to predict outbreaks of *F. hepatica* and *H. contortus* with greater precision and on a much finer spatial scale than other models present in the literature.

The Bayesian models developed allowed correction for preferential sampling arising from incompleteness of list of farms in some pilot areas for several reasons including confidentiality issues. Latent factor modelling provides a flexible approach when many climatic and environmental covariates are simultaneously considered without the risk of potential distortion due to covariate selection [2].

Climate-based spatio-temporal predictive models were developed to assess the impact of future global changes on the prevalence of *Fasciola hepatica* and *Haemonchus contortus* in ruminants in *Europe*. As regards *F. hepatica*, a model based on the Ollerenshaw Mt index was developed and validated for England and for the entire Europe using historical prevalence data as well as updated data collected during the project. Climate observations from a variety of sources and an ensemble of calibrated climate change scenarios were used. Forward projections were made for 2020’s, 2050’s and 2080’s using four different emission scenarios. The results suggested that for England, using the most extreme emission scenario, by the 2080’s, the transmission season for liver fluke will
change from the current period May to October and be extended to March to November (Fig. 3.4). The pattern of change for northern Europe is similar to that described for England, with an extension of the transmission season by up to five months. For southern Europe, projected climate change will affect winter transmission, supporting development of the free living stages of *F. hepatica* between November and March (Fig. 4.1.3.4) [4].

![Fig. 4.1.3.4 Fasciola hepatica. Change in Mt seasonality for (a, c) Northern Europe [48° N-59° N, 12° W-18.5° E] and (b, d) Southern Europe [35.5° N-44° N, 10° W-18.5° E]. The black solid line shows the *F. hepatica* Mt model driven by the EOBS climate observations (1971-2010 average). The blue lines show the Mt model driven by 5 climate models over the same time period. The orange and red lines respectively show the Mt model driven by 5 climate models under the rcp2.6 and rcp8.5 emission scenario. The solid line depicts the ensemble mean and the dotted lines depict the min and max within the model ensemble to assess the relative uncertainties. For full details see Reference [4].](image)

For *H. contortus*, a dynamical Qo model was developed to predict the propensity of the *H. contortus* population to increase, given prevailing host and climatic conditions. Development, migration and mortality rates of pre-infective stages depend on temperature and rainfall as demonstrated in WP2. The aim was to identify periods of increased risk of transmission from climatic data. The results suggested that most of France, the Benelux, north-western Germany, Denmark, northern Switzerland, most of Italy, western and southern UK, the Mediterranean islands, Poland, Austria and parts of Romania are climatically suitable regions for *H. contortus*. Interestingly, the simulated risk increases during the 2000s over most of central/north-western Europe (southern coasts of Scandinavia, Benelux, Germany, Denmark, Poland, France and the UK). Similar spatial trends were simulated for future scenarios. Focusing on changes in seasonality, slight changes are simulated for southern Europe for the future. For northern Europe, climatic conditions are simulated to be highly suitable for the transmission of *H. contortus* between May and October for the recent context. For the future, the transmission season is simulated to increase to May-December for the lowest emission scenario and to April-January for the highest emission scenario. A slight decrease in the risk is simulated during the warmest summer months (e.g. July-August) if we consider the most extreme emission scenario for the 2080’s (Fig. 4.1.3.3.5).

![Figure 4.1.3.3.5 Haemonchus contortus. Change in simulated Qo seasonality for (a, c) Northern Europe [48° N-59° N, 12° W-18.5° E] and (b, d) Southern Europe [35.5° N-44° N, 10° W-18.5° E]. The black solid line shows the Qo model driven by the EOBS climate observations (1971-2010 average). The blue lines show the Qo model driven by 5 climate models over the same time period. The orange and red lines respectively show the Qo model driven by 5 climate models under the rcp2.6 and rcp8.5 emission scenario. The solid line depicts the ensemble mean and the dotted lines depict the min and max within the model ensemble to assess the relative uncertainties.](image)
A systematic review of peer-reviewed literature was undertaken to show the spatial distribution of anthelmintic resistance (AR) in ruminants in Europe. A GIS was constructed using the georeferenced reports (at country level) of AR to the major genera of GI nematodes (Teladorsagia, Trichostrongylus, Haemonchus, Ostertagia and Cooperia) infecting farmed ruminants (sheep, goats and cattle) in Europe [5]. Reports of AR, assessed primarily using faecal egg count reduction test, were reported in all five genera of GIN and in 16 countries of Europe. The findings showed that resistance to benzimidazoles, levamisole and macrocyclic lactones is widespread in sheep and goats in Europe. Multiple drug resistance in the three main genera (Teladorsagia, Trichostrongylus and Haemonchus) infecting goats and sheep was reported in 10 countries, and monepantel resistance was recorded on a farm in the Netherlands. A number of potential sources of bias were identified in the dataset, which prevented further analysis and inference, including publication bias, lack of standardized methods for detection of AR and sample selection bias. In the majority of cases the geographic location of the record of AR was not reported in sufficient detail for ecological niche modeling highlighting the urgent need to conduct more spatially explicit studies of AR throughout Europe.

Conclusions
WP3 delivered guidelines for standardized and harmonized cross-sectional surveys of helminth parasites in ruminants. This allowed for the development of updated prevalence maps and multi-scale spatial models of the occurrence and abundance of gastrointestinal nematodes (sheep) and liver fluke (cattle and sheep) in Europe including projected changes of parasite distribution under different climatic scenarios. The results enabled to produce the first Pan-European map of F. hepatica infection risk in dairy cattle, which will serve as a milestone for further work. The current maps and the climate impact studies demonstrated that both F. hepatica and H. contortus will present an increased risk to the health, welfare and productivity of ruminants throughout Europe with an extension of their spatial occurrence and of their transmission periods. These scenarios have the potential to increase the frequency of prophylactic treatments to control helminth infection and may lead to increasing levels of resistance to anthelmintic drugs. In such scenarios, improved and tailored control programmes at multi-scale levels will become a fundamental issue to be seriously considered by animal health authorities.

Practical outcomes
The updated data and maps of the spatial distribution, prevalence and abundance of gastrointestinal nematodes and F. hepatica in sheep and cattle across Europe have great potential to deliver improved advices on control of helminth infections to veterinarians, stakeholders and farmer associations. The spatial sampling guidelines and the centralised GIS-based spatial data archive constructed during the project will guide future epidemiological studies aimed at parasitological surveillance at different spatial scales. The statistical approaches developed within the project were also successful to model the spatial occurrence of helminths throughout Europe taking into account projected changes of parasite distribution in Europe due to climate changes and spatial distribution of anthelmintic resistance in ruminants in Europe.

Scientific output and references
4.1.3.4. WP 4 - Management and sustainable control of helminth infections in European livestock farming

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**Background**

Current chemical-based approaches to helminth control utilise frequent whole flock/herd treatments, even though these are known to lead to an increased rate of development of anthelmintic resistance. Previous studies in the EU FP6 project PARASOL have established proof-of-concept for refugia-based anthelmintic treatment strategies and have demonstrated the benefits of optimised anthelmintic usage in maintaining animal performance and drug efficacy. Targeted treatment (TT) strategies i.e. optimised whole flock/herd treatment, have been shown to be beneficial in controlling nematode infections, in both large and small ruminants. Targeted selective treatment (TST) strategies i.e. individual animal treatment, have been shown to reduce anthelmintic usage in small ruminants, whilst maintaining animal production and drug efficacy. However, to date, TST approaches have rarely been studied in cattle and neither approach has been applied to liver fluke infections. The wide-scale uptake of these strategies can only be achieved with a full understanding of their potential costs and benefits; which has also not yet been evaluated. Moreover, the implementation of TST approaches is likely to greatly benefit from the availability of robust, reliable and user-friendly decision support systems and work is required to explore how such systems can be integrated into routine herd management software.

**Objectives**

To optimise the use of anthelmintics to maintain ruminant production and anthelmintic efficacy in order to provide sustainable livestock production systems. This will be achieved by (i) the development and/or validation of novel TT and TST approaches for farmed ruminants; (ii) the assessment of farm-level economic implications of these novel approaches and (iii) the integration of optimised anthelmintic usage protocols in herd management software.

**Materials and methods**

For the first objective, 10 field studies were organised in 5 countries to evaluate TT and TST approaches in different production systems and for different parasites (gastrointestinal nematode and liver fluke infections). In lamb production, production efficiency of a sentinel group and FAMACHA© were evaluated as parameters for anthelmintic treatment decisions. In dairy sheep, treatments were targeted based on the timing in relation to parturition, faecal egg counts, milk yield and number of born lambs. In dairy and beef calves, weight gain, body condition scoring and faecal egg counts were used. Finally, in adult dairy cows, age, parasite-specific antibody levels in individual milk samples, body condition score and season were evaluated. Our studies include the first evaluations of the TST principle for liver fluke control and provide evidence of the production benefit/loss compared with no or neo-suppressive control strategies. The results on production impact from field trials are used to feed herd-level economic cost-benefit models of anthelmintic treatment approaches. One model assessed the cost/benefits in dairy cattle and one in lamb production. In addition, a new framework was developed to assess the economic impact of helminth infections in the whole-farm economic context. In order to integrate TST principles in dairy herd management software a data collection and storage platform (Dairy Data Warehouse, DDW) was developed. In parallel a number of software application based on TST principles were developed and focus group meetings were organised in order to know the user needs.
Results
Ten field studies were organised in 5 countries to evaluate TT and TST approaches in different production systems and for different parasites (gastrointestinal nematode and liver fluke infections). An overview of the different field studies is shown in Figure 4.1.3.4.1 The work conducted has developed and/or evaluated several practical methods which can help to optimise anthelmintic use on farm. For lambs, a novel TT strategy for grazing lambs was developed, whereby anthelmintic is administered by monitoring only part of the flock (20%). In dairy ewes in Italy, TST indicators such as milk yield, FEC or number of lambs born were effective. However, as little anthelmintic resistance is present in this region, a TT approach, based on the timing of parturition (with regular efficacy checks to monitor resistance development) will be promoted. In growing cattle, weight gain and body condition scoring combined with faecal egg counts were successful TST indicators. In adult dairy cattle, anti-helminth antibody levels have value in targeting treatments to individual animals, especially for liver fluke, but could be further refined by combination with other parameters such as age or body condition score. The studies provide a strong evidence of the production benefit of optimized anthelmintic treatment approaches. TST approaches can have a clear production benefit to the farmer, while they are considered to preserve anthelmintic efficacy. Moreover the TST concept can be adapted according to region, farm and helminth-specific factors making it more acceptable to farmers. Nonetheless, the studies also pointed to potential problems that can be associated with TST approaches, for instance the lack of effect on pasture infectivity levels. The results generated in this task were used to feed economic cost-benefit models of anthelmintic treatment approaches. All deliverables and milestones were achieved.

TT and TST approaches can offer advantages over traditional whole-herd treatment approaches from economic point of view. The most suitable TT/TST indicators will allow pen-side decisions, which make cost effective decisions, and these factors will be critical for uptake, especially in cattle. In order to determine the economic impacts of TT/TST, cost-benefit studies were conducted for cattle and sheep. In dairy cattle, 4 different treatment strategies were compared with a baseline situation where no treatments were applied: whole-herd at calving (S1), selective at calving with (S2) or without (S3) treatment of the first-calf cows and whole-herd when animals are moved from grazing to the barn in the fall (= “housing” treatment, S4). The benefit per lactation for an average dairy herd varied between € -2 and € 103 (average € 50) for S1, € -2 and € 101 (average € 49) for S2, € -14 and € 82 (average € 34) for S3 and € -33 and € 57 (average € 12) for S4 (Figure 4.1.3.4.2). The farmer’s risk associated with any treatment strategy, as indicated by the width of the 95% credible intervals of economic benefit of anthelmintic treatment, decreased with herd size and with increasing level of exposure as assessed by bulk tank milk ELISA. The order of the different strategies when sorted by expected benefit was robust to changes in economic input parameters. On average, strategies applying anthelmintic treatment at calving outperform the strategy applying treatment at housing. Within the strategies applying treatment at calving, more selective treatment strategies can be economically sustainable, especially in farms with a low or medium level of infection although practical, low-cost indicators for treatment decisions are still needed to support their wider application in the field.
In lamb production in the UK, over four years of trials comparing groups of lambs subjected to TST
based on production efficiency, SPT (strategic prophylactic treatment), metaphylactic treatment (MT) and NST (neo-suppressive treatment), average daily live-weight gain was not significantly lower in TST compared with NST groups, but number of treatments was reduced by around half, and the efficacy of ivermectin was higher at the end of the trial in TST than NST groups, as assessed by % faecal egg count reduction. On farms trained in the use of pen-side faecal egg counting (FEC) technology, the number of treatments given to growing meat lambs was reduced, while labour costs also decreased on around half of the trial farms. Labour savings due to reduced number of treatments was offset by increased time spent monitoring infection levels using FEC. Net savings were positive on 7 of the 8 farms, using a labour rate of €12 per hour.

While we used existing economic techniques to assess the effects of TT and TST, there was also need to develop a new analytic framework. Current helminth disease cost estimations are based on partial and ‘expected’ rather than ‘observed’ records and thus do not provide insights into the effect of a helminth infection on the whole-farm economic performance. Moreover, if we want to use economic assessments for decision support at farm level, economic impact estimations must be farm specific. We developed a new approach, based on efficiency analysis to assess the impact of GI nematode infections on farm performance. Efficiency analysis studies the conversion of input(s) into output(s) and compares the current performance level of a farm with the performance level of peer farms with similar production technologies. Using this approach, a negative relationship between antibody levels to GI nematodes in bulk tank milk and technical efficiency was identified. GI nematode infections appeared to mainly constrain the efficient transformation of pasture, health related costs and labour into milk. In addition, the GI nematode-associated inefficiency was mitigated when higher levels of concentrates and roughage were supplied. Towards integration of GLOWORM knowledge in herd management software. Firstly, the need to harmonize and improve the quality of cow and farm management data was recognized. A long list of conversion programmes has been developed in order to store data from different farm management systems in a uniform way and on a central database, called Dairy Data Warehouse (DDW). In addition, data quality control mechanisms have been developed. The DDW now has become a commercially available product. It went “live” on Oct 1st, 2014 for veterinarians and dairy consultants for use in their daily consulting work. Secondly, now that high quality farm management data are more easily accessible, different applications using these data and supporting smart management decisions need to be developed. Milkbot, a new data-analysing tool to assess the effect of disease information or interventions on milk production, supporting targeted anthelmintic treatment has been integrated within DDW. The MmmooOgle system is a data capture and presentation platform for smartphones under development that should allow the capture of on-farm “boots in the manure” data and combined with data from the DDW will generate reports to support management decisions. Other tools that were further developed are (i) ParaCalc\textsuperscript{6}, an open-access platform offering tools to estimate the economic cost and support the control of helminth infections on dairy farms (Figure 4.1.3.4.3); (ii) a web interface to evaluate anthelmintic efficacy based on faecal egg count data (see also WP2) and (iii) a thermal-humidity index anomaly detection process to alert for heat stress.
Finally, focus group meetings revealed that farmers seem more willing to embrace new smart phone technologies than veterinarians. The main requirement of farmers was that software to support helminth control should be able to fit in an all-round technology where all data of their herd is implemented and visible. If such a system were available, we expect the adoption would be positive. For the private veterinarian, the importance of linkage with his billing system emerged, so that advice delivered can be properly accounted for. The time-managing component was important for both groups; ‘a new application should be able to end a day’s work in the stable’. All kind of data should be available on the spot.

Conclusions
TST approaches can have a clear production benefit to the farmer and preserve anthelmintic efficacy. Moreover the TST concept can be adapted according to region, farm and helminth-specific factors making it more acceptable to farmers. Economic analysis showed that TT or TST approaches are competitive and sometimes superior to traditional whole-herd approaches. However, given the large variation in possible benefits within each treatment strategy for different farm situations, decision support systems are needed to take into account the multitude of cow, epidemiological and economic factors that determine the economics of nematode control and select the optimal treatment strategy for a specific farm. The user needs for such decision support systems are very different for veterinarians and farmers. By the use of focus group meetings, the development of a common database platform and several software applications, a strong base has been created for integration of TT/TST principles in user-friendly software applications for both professions.

Practical outcomes
The work conducted has developed and/or evaluated several practical methods which can help to optimise anthelmintic use on farm. For lambs, a novel TT strategy for grazing lambs, whereby anthelmintic is administered by monitoring only part of the flock (20%). In dairy ewes, although TST approaches were promising, where there is little anthelmintic resistance present, a TT approach will be promoted, with regular efficacy checks to monitor resistance development. In cattle, TST methods were evaluated based on weight gain, FEC in combination with BCS and helminth-specific antibody levels in individual milk samples and appear to show promise. Economic data are now available and can be used to promote the uptake of TT and TST approaches. The developed economic models can be used to increase the farm-specificity of the given worming advice and to better integrate this advice in the whole-farm economic context. In dairy cattle, we found that bulk-tank ELISA can be a suitable decision criterion for the treatment approach: TST approaches are most economic when used in moderately infected herds (like it is mostly the case in cattle in Europe) whereas in the highly infected herds whole-herd treatment approaches remain most economic. In sheep, we provided strong evidence for the immediate economic benefits of applying TT/TST on commercial sheep farms, even before the medium term benefits of protected drug efficacy are estimated. Several software-applications were developed (Dairy Data Warehouse, Anthelmintic Efficacy Calculator or ParaCalc®) & are already accessible by veterinarians & farmers.
Scientific output and references:

4.1.3.5. WP 5 - Dissemination, training and exploitation of results

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Background
The dissemination of results from the project is essential to ensure that everyone is aware of the impact of global changes on the epidemiology of helminths infections and that urgent action needs to be taken to ensure the future viability of livestock production. The dissemination platform consisted of (i) a web-site, serving as both a database resource for project partners and a dissemination gateway to the wider scientific and end user community and (ii) well balanced communication actions to relevant stakeholders and press releases to confirm the dissemination strategy.

The project brought together the expertise from 14 European participants involving a multitude of disciplines such as parasitology, epidemiology, molecular biology, farming systems and GIS. Partners and young scientists in particular benefited from these expertise through staff exchanges to member laboratories and several dedicated workshops held through the life time of the project. This also allowed for the use of standardised tools and tests across the consortium to ensure optimal implementation of the project. During the GLOWORM project several novel diagnostic tools to evaluate the infection status of herds, flocks and individual animals and that could be used in the development of effective treatment/control options to the farmer have been developed. In addition as a vast variety of geographic and climate data in combination with prevalence of helminth infections within different EU countries and regions and different farm animal species and production systems have also been collected. These data provides the opportunity to develop additional tools for the management of endemic diseases through incorporation into herd management software packages for the more rapid and efficient management of livestock diseases.

Objectives
To (i) maximize the international visibility of the project through developing a dissemination strategy focused towards different target groups; (ii) to improve capacity and knowledge through specialised training courses and (iii) to exploit project results into existing software packages and
novel diagnostic assays and iv) to disseminate knowledge generated within GLOWORM to end-users (farmers, farmers associations, veterinary professionals, etc.).

Results
A dedicated GLOWORM website, http://www.GLOWORM.eu, was created under Drupal, a content management system allowing editing via multiple users. The website includes static content (project description, consortium presentation) and more dynamic items such as the news section which has been regularly updated with news and information and served as the main communication portal with the general public and end-users. The website was monitored for usage since June 2013 using Google Analytics. In total 3,761 visitors initiated 4,593 sessions totalling 12,445 page views. On average the visitors looked at 2.71 pages per session, which means that they not only visited the main page but also were active with other content. The session time was on average 1:34 minutes. Returning visitors (18%) spend longer time on the site; up to 3 minutes. People from all over the world were interacting with the site. The top three countries were UK, Belgium and Germany, countries included in the consortium. However, in the fourth place is USA, demonstrating that the site attracted people beyond the consortium.

Press releases were posted on the GLOWORM website: http://www.GLOWORM.eu/press and on the CORDIS website: http://cordis.europa.eu/fetch?CALLER=EN_NEWS&ACTION=D&SESSION=&RCN=34462. Press releases were also send to all partners for translation into local language where applicable and/or adapted for the local situation. The members of the consortium have been very active in the dissemination of research results through scientific publications, conference and poster presentations, articles published in popular press, general presentations and workshops (Figure 4.1.3.5.1). In addition talks/seminars/workshops were delivered to over 500 farmers/veterinarians in most participating EU countries involved in the project.

Staff exchanges, mainly targeting young scientists (master and PhD students), were established between member laboratories. Several training workshops were organised: (i) ‘From experiment to model’ related to mathematical modelling and included specific formal training in software packages ‘Poptools’ and ‘R’. (ii) Two workshop on Geographical Information Systems (GIS) and the develop models for the spatial distribution of *Fasciola hepatica* in dairy cattle in each of the GLOWORM pilot areas selected in Belgium, Germany, Ireland, Poland and Sweden. All material was provided to the participants via the online distance-learning platform (learning.avia-gis.com). This platform was also used for forum discussions and the provision of extra ancillary data. (iii) Two workshops on Spatial Analysis with the general aim of to train GLOWORM partners to perform spatial data analysis, hypothesis testing and prediction using different statistical packages. During the GLOWORM project several diagnostic tools have been established. In addition a vast variety of geographic and climate data in combination with prevalence of helminth infections within different EU countries and regions and different farm animal species and production systems have
also been collected. Due to the combination of skill of the parties involved algorithms of combined info delivery have been developed or are in a process of development. Different marketing potentials are seen according to the different tools developed: (i) The Luminex assay (single or multiplex antibody detection) - kits will be limited to specialized laboratories using a Luminex platform or that will be willing to invest in such technologies. Benefit for the end user will certainly be the automation, lower cost and fast turnaround time in obtaining results. Governmental laboratories are mainly seen as the end users of this technology. (ii) Great potential is seen in development of either ELISA tests or on-site tests by industry partners using reagents/antibodies developed in GLOWORM. A high demand of on-site tests to detect helminth infections exists with farmers/veterinarians as end-users. (iii) During GLOWORM various algorithms for risk assessment and decision making based on prevalence (results of laboratory test), climate data, geologic data and herd management information was developed. These applications could be used by industry as marketing tool to optimize treatments as well as actions for disease control. End users would be farmers and veterinarians as well as animal health services.

The user and system requirements to develop a resource-based sampling tool have been developed. This has been reflected in updated system architecture of VetgeoTools. The tool includes a resource allocation method, where the user can enter the budget and verify the impact of that on the accuracy that he can maximum achieve with that budget. The user is presented with a wizard guiding towards the correct sampling tool (spatial estimation of population statistical parameters, spatial prediction, and sampling for moving objects) and a provided with a description of the basic assumptions for each algorithm used (Figure 4.1.3.5.2)

Conclusions
During the project appropriate measures were taken to engage with the stakeholders (farmers, veterinarians, policy makers and researchers) about the project and to create awareness of the goals of GLOWORM and to maximize the international visibility of the project. A number of farming events and stakeholder workshops/seminars were attended to ensure that stakeholders understand the objectives of GLOWORM and were provided detailed information and advice on the sustainable control of helminth infections in a changing world. A dedicated project website has been maintained that also provide up to date information to end-users. In addition several scientific papers have also been published in peer reviewed scientific journals, many of them Open Access journals. Great potential is seen in the use, by industry partners, of the different diagnostic reagents/assays and data algorithms/tools in the development of herd management information systems.

Practical outcomes
• Establishment of dedicated GLOWORM website
• Successful training course held in GIS and Spatial statistics
• Dissemination of results through peer reviewed scientific publications and presentations at national and international conferences
• Dissemination to end-user groups in key regions through a number of successful farming events
### 4.1.3.6. WP 6 - Coordination and project management

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**Objectives**

To provide GLOWORM with an efficient management structure supporting the members of the consortium towards a successful realization of the project’s objectives within the foreseen timescale and available resources with the following three principal management tasks: (1) productive collaboration and integration of all partners, (2) monitoring of progress and (3) completion of EC requirements.

**Project management**

The main administration of the project has been undertaken by the team of the coordinator P1. The coordinator was also supported by the EU-Cell team of Ghent University. Communication on progress of the work was mainly done with the WP leaders by mail. In order to facilitate efficient communication and exchange of documentation within the consortium, a consortium-owned website was developed. The GLOWORM dedicated website, [http://www.GLOWORM.eu](http://www.GLOWORM.eu), was established within the four first months of GLOWORM and has been regularly updated since. To ensure data protection some of the information provided the website has a confidential part that is only accessible to GLOWORM members.

Organisation of General Assemblies (at the start, mid-term and end of the project) and Steering Committee (SC) meetings (every nine months)

Five meetings have taken place during the project period:
2. Bristol - Steering committee and selected partners: October 8 - 10 (2012)
4. Napoli - Steering committee and selected partners: 31 March – 1 April (2014)
5. Ghent - General Assembly: December 15 – 17 (2014)

**Identification of members of External Advisory Group**

An External Advisory Board (E-AB) was established to oversee the progress of the project. The E-AB has given advice on project execution, dissemination and training needs and have supported the consortium in the advocacy of GLOWORM policy recommendations. The E-AB attended all meetings of General Assembly and SC. They have had access to the full project proposal and progress reports and provided independent comment on progress and project integration. The 3 members of the E-AB were Prof. Dr. Adrian Wolstenholme (Dept. of Infectious Diseases, College of Veterinary Medicine, University of Georgia, US); Prof. Dr. Frank Jackson (Moredun Research Institute, Midlothian, UK); Dr. Darren Shaw (Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK).

Ensuring that the information resulting from the project was effectively disseminated to researcher, stakeholders and user communities.

All research groups were linked through a common address list to enable efficient internal communication flow by phone, e-mail, Skype or personal meetings. This was completed by an internal web-based platform for data and information exchange and other online services. Clear vertical communication channels for reporting and monitoring were established for the scientific steering of the project. These channels led from the scientific base of all researchers via the WP Leaders to the SC. Horizontal interactions between the different WPs were established through several participants being active in more than one WP and the fact that the activities of the WPs strongly depended on each other.

**Enforcing compliance with regulatory and ethical regulations and guidelines, including gender issue. Progress monitoring & risk management. Reporting to the European Commission.**

The Co-ordination Team and the WP leaders continuously monitored the project’s progress on the basis of the Gantt chart presented and ensure timely submission to the European Commission of a full progress report at months 18 and 36 (Final report).
4.1.4. The potential impact and the main dissemination activities and exploitation of results.

4.1.4.1. Potential Impact

Novel low cost multi-species diagnostic tools.
A range of multi-parasite diagnostic tests (including devices and techniques), using faeces, blood and/or milk samples to identify and quantify helminth infections in flocks/herds or individual animals, have been developed within GLOWORM. DNA tests were also developed to understand anthelmintic efficacy in nematodes of both cattle and sheep and applied in key locations within Europe. These new tests offer many advantages over current tests in terms of time, labour, ease-of-use and sensitivity. For example, the developed Luminex® assays provide a platform for rapid, high-throughput, multi-species tests to be applied to the same sample set. Many of the techniques developed, can be readily implemented by research laboratories. This will facilitate increased understanding of the impact of helminth infections amongst farmers, practitioners and other stakeholders. Apart from the development of new tests, existing and widely used techniques were improved significantly. For example, faecal egg count (FEC) techniques were improved in terms of sensitivity and ease of application e.g. through development of the Mini-FLOTAC device and composite faecal sampling protocols. Compared to the traditional individual animal sampling techniques, composite (pooled) sampling is much less laborious to carry out and significantly cheaper. For example, since being introduced by government veterinary laboratories in the UK, over 2,000 composite tests have been conducted. Analysing these samples using single tests would have cost farmers £193,812 (based on the 2012 AHVLA price list), but the composite test cost them only £52,439 – a 74% saving (White, N., Information enquiries manager, AHVLA, 2014). As well as this direct reduction in monitoring costs, farmers save in terms of treatment costs as FEC monitoring can lead to a decrease of as much as 75% in the number of treatments given to lambs (Meat Promotion Wales, 2011). In GLOWORM, further development and validation of pooled FEC protocols will underpin wider uptake in Europe with up-scaled economic benefits. These tests can now be applied to assess helminth infection intensity and to determine anthelmintic efficacy. Reducing cost and time ‘barriers’ to the uptake of these parasitological tests will improve the frequency of their application on farms. These techniques make use of existing laboratory infrastructure and are, therefore, directly applicable now. Through the addition of an online tool to determine anthelmintic efficacy by faecal egg count reduction test (FECRT), the interpretation of FECRT, which was previously the subject of much scientific debate, is now also brought up to statistically robust standards. This will help veterinarians/consultants to deliver high quality advice, based on sound science.

Provision of spatial and temporal disease risk maps
Multiple new robust models of parasite abundance and disease risk were developed and validated, some focusing on the seasonality of disease risk (1, below) and others on spatial distribution (2, below) in Europe. The impact of this work was significantly enhanced by an exchange of knowledge and modelling expertise with, for example, temporally-explicit models being applied within a geographical information system (GIS)-based environment. These exchanges will have lasting impact within European modelling communities, facilitating the modelling of changing seasonality of infectious diseases in specific locations.

1. Parasite transmission models
As the climate in Europe is changing, and farm management is changing with it, it is pertinent to know how the seasonality of helminth disease risk will change and how disease risk can be minimised in the future. A new differential equation model framework of the free-living stages of the target helminth species, driven by both climatic data and farm management factors, was
established. GLOWORM both united, and significantly expanded on, published experimental efforts and built new models from the ground up. For species such as *Cooperia oncophora* and *Fasciola hepatica*, GLOWORM provided the first models of this kind. Moreover, systematic reviews were used to populate models of the parasitic phase of the most important nematodes. For the first time, it is now possible to link and predict the combined climatic, host-associated and farm management factors on helminth populations.

Importantly, this type of mechanistic life-cycle modelling forces the researcher to identify and address important gaps in knowledge in systematically-based research. This helped focus experiments and yielded significant advances in our understanding of the effects of, notably, temperature and rainfall on parasite populations, their interacting effects, and how farm management can be improved to mitigate increased disease risk. GLOWORM enhanced our understanding of the effects of temperature on parasite populations and seasonality, and, importantly, explicitly incorporated the effects of rainfall and changing rainfall patterns in mechanistic models for the first time. It also provided an EU database quantifying the important farm management factors, such as stocking density and grass growth seasonality, at the EU scale and made this information available for modellers and the wider research community. Furthermore, the economic impact of helminths affecting ruminants was quantified, facilitating estimations of future costs to the industry associated with enhanced worm burdens, and providing a basis for choosing economically optimal control strategies.

GLOWORM has, therefore, provided a framework for predicting (1) future changes to parasite populations, (2) how these can be mitigated and (3) whether new strategies are likely to be economically beneficial. The developed models have already been applied in the field in the form of real-time disease risk maps. Work on seasonal changes in *Nematodirus battus* risk due to climate change has improved spring disease forecasts (http://www.scops.org.uk/news-detail.php?NewsID=21, accessed 20 July 13). An online *N. battus* risk forecast was launched by SCOPS (UK Sustainable Control of Parasites in Sheep initiative) in 2013 and accessed by more than 19,000 web users (of whom 60% new users), twice the number normally visiting the SCOPS website in this period (SCOPS website google analytic report, Sept 2013). Users on average accessed 2.74 web pages, demonstrating increased access to advice on sustainable parasite control as a result of the forecast provision.

Funding applications have been submitted to further application of the GLOWORM models to practical parasite control (see below). Simulations of the impact of alterations in the timing of anthelmintic applications will directly inform the industry on future worming strategies; GLOWORM has provided a platform to roll these out to the industry, for example, through the development of decision support tools by SMEs within the project. The GLOWORM nematode models formed important components of successful applications for further research funding, including from UK government (InnovateUK and BBSRC) to integrate improved predictions of pasture infectivity into pen-side FEC technology (AutoFEC project: €802,000), and to extend them to consider the spatial distribution of refugia on farms (BUG consortium, €3,900,000; https://bugconsortium.wordpress.com/). These projects aim to apply the models to produce practical decision tools for farmers to manage pasture grazing for reduced and sustainable anthelmintic use.

2. Multi-scale spatio-temporal modelling

Validated and standardized sampling and diagnostic procedures (from the farm to the lab) have been developed within GLOWORM to conduct cross-sectional surveys of helminths in sheep and cattle in Europe. Gastrointestinal nematodes and liver flukes were studied in order to: (i) construct state-of-the-art parasite abundance maps, to be disseminated to practitioners, farmers, stakeholders and decision-makers and (ii) provide high-quality data, gathered within the project, as input for the multi-scale spatial models. Therefore, a multi-scale spatial modelling approach was used, combining Bayesian and other statistical approaches with field parasitological data collected using the developed protocol as well as the compiled extensive set of climatic, environmental and socio-
economic data as an input. These methods were used to model the spatial occurrence of sampled parasites in each pilot area and to conduct a comparative analysis of parasite occurrence between these areas distributed throughout Europe. Other model outputs forecast the impact of global change (in particular climatic changes) and anthelmintic resistance on parasite occurrence in Europe for selected helminth species (e.g. *Fasciola hepatica* and *Haemonchus contortus*). An extensive standardized database of parasitological records gathered by the GLOWORM partners prior to and during GLOWORM, and not available beforehand, will remain available to the consortium for future use and expansion, providing a much-needed benchmark for model validation and for the prediction/assessment of future changes.

These updated data and maps of helminth abundance in sheep and cattle across Europe are directly applicable, practical regional/local risk assessment tools and will be used by veterinarians, farmer associations and other stakeholders to deliver improved advice on the control of helminth infections in large and small ruminants. The statistical methods developed within GLOWORM also have a legacy as they are essential for modelling the spatial occurrence of helminths in each EU region and to conduct a comparative analysis of helminth occurrence (as well as anthelmintic resistance status) between areas in Europe.

**Advantages of the inclusion of SMEs.**

The inclusion of four SMEs within GLOWORM has resulted in several benefits which directly contributed to the overall success of the project. Crucial advantages, in no particular order, include:

1. In general, SMEs provided academic partners with important insights into business context, for example, on protocols for rolling out newly developed test(s). Similarly, SMEs greatly enhanced the awareness for the need to bring findings to the field.
2. SMEs were able to provide commercially available state-of-the-art training courses to participants, for example, an excellent GIS modelling course run by Avia-GIS and state-of-the-art Bayesian statistics course run by E&P.
3. Avia-GIS, with sound experience of the development of modern websites, was also able to provide the project with both a state-of-the-art website and ways to monitor its use. The professional site certainly increased in popularity amongst users and became an important part of our dissemination activity.
4. The client networks of SMEs in the field, most notably Laboklin, provided the consortium with access to samples in regions (for example, Poland) which would otherwise not have been accessible.
5. The high-throughput facilities of Laboklin facilitated analysis of gathered samples at a scale and efficiency the project would otherwise not have been able to provide.
6. The Dairy Data Warehouse, specialising in making robustly analysed data readily available to farmers, in an easily-understandable format, through the development of mobile phone apps, provided the consortium with ideas and know-how in this rapidly developing field.
7. Avia-GIS and the Dairy Data Warehouse provide the academic partners with decision support tools through which important findings, for example optimised anthelmintic use principles, may be rolled out to the industry.

GLOWORM, therefore, provided a functional platform for symbiosis, with SMEs profiting directly from research findings and academic partners benefitting from their know-how of methods to apply new research to field situations. As a result of the inclusion of SMEs, in addition to the scientific output, GLOWORM has generated useful tools for farmers, veterinarians, laboratories and/or research institutions. These tools have become available on a dedicated platform and will remain accessible to the general public beyond the project to ensure sustainability.

**Bolstering sustainable livestock production and optimising the use of pharmaceutical resources.**

GLOWORM has developed a package of tools, strategies and underpinning knowledge that will be
used to improve the control of gastrointestinal nematodes and liver flukes in grazing livestock. Many of these will also be applicable to livestock producing countries outside of the EU. The systematic review of the economic effects of helminths on livestock production in Europe provides the basis for optimised intervention strategies, as well as the evidence required to place helminth diseases in their proper economic context when making strategic policy decisions on disease control. The novel tools developed to diagnose and identify worm infection will improve the ability to understand exactly which helminth parasites are contributing to disease, and therefore ensure that the correct treatment is administered. The improved FEC methods and sampling strategies will help to ensure that these tests are more frequently used by both veterinarians and farmers. Coupled with knowledge on new anthelmintic treatment strategies and their economic impact, this provides farmers and their advisors with much improved decision making on anthelmintic treatment strategies. The transmission models developed are arguably the only feasible tools capable of both improving our understanding of key drivers of parasite abundance in future and designing new intervention strategies which can then be tested in the field. These models are already being used to improve current anthelmintic use by optimising the timing of drug doses for maximum impact on parasite populations. They have also already been exploited to provide real-time risk maps for disease caused by *Nematodirus battus*, a major cause of mortality and morbidity in young lambs in the UK (see above). The maps were delivered in partnership with the UK sheep industry and are freely available to farmers online (www.scops.co.uk and http://www.nadis.org.uk/parasite-forecast.aspx.) GLOWORM studies conducted to determine optimised use of pharmaceutical agents has resulted in the promotion of evidence-based advice to farmers about how best to use these products in a range of farming situations.

Taken together, the outputs from GLOWORM, including the novel tools, optimal and robust sampling strategies, risk maps and evaluation of different anthelmintic treatment strategies, will help to work in concert to produce more efficient livestock production with optimised intervention. This will, by default, reduce the dependency on drug applications and, therefore, enhance food security in the face of global change. The novel incorporation of climatic stochasticity, along with explicit drivers of farming systems, in models will enable the design of farm practices that are robust and responsive to increased variation in climate, including unpredictable events. Thus, a key and novel impact of GLOWORM will be to provide the tools for proactive intervention at policy level to increase the resilience of European farming systems to increased disease risks arising from global change.

**Applying knowledge gained to reduce environmental footprint of livestock products.**

Many of the outputs from GLOWORM can be used to reduce the parasitic burden present in the livestock industry in the EU, which was one of the aims of the project. We have also delivered methods which optimise treatment strategies, therefore increasing efficiency within the livestock industry. The effective management of disease will also help to reduce the carbon footprint of the livestock industry. By ensuring that only animals affected by helminths receive treatment, the number of drenches administered can be significantly reduced, also reducing the impact of these chemicals on the environment and their residues in meat and milk products destined for the human food chain. Furthermore, an additional benefit of optimising anthelmintic use is the ability to preserve anthelmintic efficacy. This will help to maximise the life-time of currently available anthelmintic drugs, and any new products that may reach the market in future, to maintain high levels of animal health and productivity. In turn, this will increase efficiency in the use of the available nutritional resources, thereby reducing the environmental footprint of livestock production.

**Training for the future**

In recent decades, we have seen the critical mass of experienced veterinary parasitologists dwindle. However, over the same time frame, the importance of the veterinary helminths as a cause of
serious economic loss has increased significantly. The young scientists involved in and trained by GLOWORM in Europe will provide new impetus to research in veterinary parasitology. This new generation will carry forward and build on the novel insights and techniques and contribute to making tools commercially available. Close collaboration with farmers in the evaluation of novel control methods may also be used as demonstrations of the advantages of these approaches and to share experiences within the farming community.

The overall success of GLOWORM, and the close collaboration it provided, is probably best summarised in the newly proposed worm platform, aiming to further the GLOWORM principles beyond the lifetime of the project. Noteworthy, all GLOWORM partners have supported the foundation of this self-funded new initiative to keep the consortium together, to exchange knowledge, research plans and apply for further funding together. Within the continuation of the consortium, training will be directed to students (PhD/MSc), practitioners (veterinarians and animal production advisors) and farmers as follows.

**Added value of interaction with other projects**

The work carried out within GLOWORM has added benefit to other EU projects, most notably PARAVAC, aiming to develop and deliver vaccines for helminths. For example, one work package within PARAVAC is addressing the likely impact of vaccine deployment on parasite epidemiology. However, this impact is strongly co-determined by the success of development of the free-living stages, which can be exclusively included in the GLOWORM models. Similarly, detailed models of the parasitic stages of the important parasite, *Fasciola hepatica*, could not have been established within the lifetime of GLOWORM but were further developed within PARAVAC; for the first time, a full-cycle model will now be able to address the host-parasite-climate-management interactions in this important parasite.

In terms of bringing GLOWORM-developed disease risk models to the field, the successful interaction with ‘SCOPS’ was described above. Further efforts to perfect the *Nematodirus* disease risk maps are underway. A UK grant application has been submitted with industry partners to make real-time prediction of *Fasciola* disease risk available to farmers in the form of ‘phone apps’. At the final GLOWORM meeting in Ghent, links were established with the Dutch Animal Health Service (GD Deventer) to combine and apply GLOWORM-established risk models with an established field-based risk prediction system and provide a more robust prediction system, initially for the Netherlands. Follow-up grants, aiming to bring other GLOWORM-developed models to the field, directly linking in with the industry (such as levy boards, milk recording companies), are in preparation. Discussions regarding uptake of decision support systems for anthelmintic use have been ongoing and a project application has been submitted to expand this approach to UK cattle farms.

**Impact of collaborations established within GLOWORM**

The three years of the GLOWORM project produced a strong and fruitful collaboration of specialists in different field of research including veterinary parasitology, production medicine, epidemiology, geostatistics, information and spatial sciences. Scientific and technology transfer between partners in the consortium has been a key example of successful collaboration, because of the strong synergism established between the different groups. Links between the academic and SME groups were encouraged with the aim to build durable research and training collaborations that will extend beyond the lifetime of GLOWORM. The lasting impact of groups working together in this FP7 (GLOWORM) and in a previous FP6 (PARASOL) has been underlined by the ongoing collaboration and scientific outputs. Furthermore, during the last GLOWORM assembly in Ghent (December 2014), future meetings of a self-funded consortium have already been proposed, showing that the consortium has been successful and understands the benefits of working together.
4.1.4.2. Main dissemination activities

Within GLOWORM, a range of dissemination activities have been undertaken (detailed in section 4.1.3, WP 5). The consortium aimed to ensure that the results were delivered directly to those people who would most benefit from the knowledge, in this case farmers and veterinarians, and so developed the webpage and delivered many talks/seminars/workshops to over 500 farmers/veterinarians in most participating EU countries involved in the project. Numerous articles were written and published in the popular press, either providing updates on the progress made within the project, or showcasing the results achieved. GLOWORM has also been able to highlight the areas of research to policy makers. As a group of scientists, the group has also ensured that the work conducted was of high scientific quality and this is reflected in the numerous presentations at national and international conferences and the publication, thus far, of over 60 peer-reviewed articles in scientific journals. The impact of these dissemination efforts is that the knowledge collected within GLOWORM has been distributed throughout the course of the project, to a wide group of end-users. Towards the end of the project, a workshop was held at Ghent University to showcase the outputs of the project. This was open to veterinarians, stakeholders and other members of the scientific community and was well attended. The outputs of the project has been and will continue to be disseminated to veterinarians, stakeholders, farmer associations, and similar organizations (e.g. COWS and SCOPS in UK and CREMOPAR in Italy) to deliver improved advices on control of helminths in Europe and leave a valuable GLOWORM legacy. The project participants also comprise key opinion leaders within their respective countries, and many are responsible for veterinary curriculum development and delivery in their host institutions, as well as in national and supra-national bodies such as the European Veterinary Parasitology College (EVPC). New paradigms in parasite control, as well as up-to-date technical detail and scientific understanding, therefore is disseminated to a large proportion of veterinary undergraduates and specialists in training throughout Europe.

4.1.4.3. Exploitation of results

It is the intention of all GLOWORM partners to fully exploit the results achieved within GLOWORM. For example:

1. We intend to maintain the webpage into the future. This will continue to provide a catalogue of the outputs of the project for the public and with contacts for further information.
2. There are several on-going discussions with commercial partners or other industry representatives regarding GLOWORM findings and the potential for commercial applications.
3. We have already published many of the findings, and intend to continue to publish appropriate scientific findings in high impact peer-reviewed journals. This will make the research findings available to other members of the scientific community and may well promote further research.
4. We have already put considerable effort into disseminating the findings of the project to the target end-users (farmers, veterinarians, policy makers) and we will continue to do so, in the hope of driving uptake of new ideas, methodologies and strategies by these communities.

Overall, the GLOWORM project has significantly increased knowledge of the impact of helminth infections and provided a range of novel tools for improved monitoring, mapping and modelling of helminth infections in European livestock. These advances will help support a sustainable, efficient and competitive ruminant farming industry in Europe and contribute to long-term food security in the face of global change.
4.1.5. Project information.

Project website: www.gloworm.eu

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