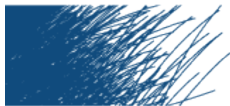
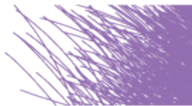

FACULTEIT DIERGENEESKUNDE
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DEVELOPMENT OF A TRANSMISSION MODEL FOR GASTRO-INTESTINAL NEMATODE INFECTIONS IN CATTLE.

SIEN VERSCHAVE

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“Don’t ever be afraid to admit you were wrong.
It’s like saying you’re wiser today than you were yesterday.”

Robert H. Newell, humorist

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List of abbreviations

A	Adult nematodes
AWB	Adult worm burden
BCS	Body condition score
CI	Confidence interval
Co	*Cooperia oncophora*
Cp	*Cooperia punctata*
Cspp.	*Cooperia* spp.
DFP	Daily faeces production
DH	Dry herbage
Dv	*Dictyocaulus viviparus*
eL_4	Larvae in the early fourth stage
epg	Eggs per gram faeces
F	Proportion of females
f	Daily faeces production
FAO	Food and Agriculture Organization of the United Nations
FEC	Faecal egg counts
FEC_n	Faecal egg count at necropsy
FSG	First season grazer
FWB	Female worm burden
GIN	Gastro-intestinal nematode
GINs	Gastro-intestinal nematodes
h	Proportion of developing pre-adult nematodes entering hypobiosis
Hc	*Haemonchus contortus*
Hp	*Haemonchus placei*
ID	Infection dose
L_1, L_2, L_3, L_4, L_5	First, second, third, fourth and fifth-stage larva

L3h	Third stage larva on herbage
L3i	Ingested third stage larva
NA	Not available
Nh	*Nematodirus helvetianus*
Oer	*Oesophagostomum radiatum*
Oo	*Ostertagia ostertagi*
P	Pre-adult nematode
p.i.	Post infection
Pa	Hypobiotic nematode
pA_f	Proportion of adults that are female
PLC	Pasture larval count
r	Acquired immunity
RE	Random-effects
SD	Standard deviation
t	Duration of infection
Ta	*Trichostrongylus axei*
Tc	*Trichostrongylus colubriformis*
TST	Targeted selective treatment
TT	Targeted treatment
U.K.	United Kingdom
δ_1	Development rate from ingested L_3 to mature adult
δ_2	Development rate from arrested L_4 to mature adult
μ_1	Pre-adult mortality rate
μ_2	Arrested L_4 mortality rate
μ_3	Adult mortality rate
λ	Daily fecundity (eggs produced)
ρ	Immune response
σ	Immune decay in the absence of exposure to infection

CHAPTER 1

GASTRO-INTESTINAL NEMATODE INFECTIONS IN CATTLE IN THE 21ST CENTURY AND THE USE OF TRANSMISSION MODELS TO SUPPORT SUSTAINABLE CONTROL PRACTICES

1.1 General introduction

Gastro-intestinal nematodes (GINs) represent an important constraint for livestock farming in temperate regions and are highly prevalent in grazing livestock. GIN infections impair the health of livestock, but due to intensive chemoprophylaxis the number of clinical infections has decreased and nowadays the focus lays mainly on the economic impact of the disease. The future control of GINs, however, is challenged by several factors such as the development of anthelmintic resistance and the impact of changes in climate and farm management. These changes affect parasite transmission and epidemiology, and enforce the need for alternative and innovative parasite control approaches. Mathematical models simulating disease dynamics have great potential to improve understanding of parasite epidemiology and to support the implementation of alternative parasite control strategies. In order to fully understand how transmission models can be a tool to deal with the expected changes of GIN epidemiology and control, it is necessary to first clarify the dynamics and underlying drivers of the host-parasite interaction. The following text explains the epidemiology and control of GIN infections and elaborates on the expected trends for both host and parasite. Further, transmission models as an asset to improve our understanding of current and future epidemiological consequences of the expected changes are discussed.

1.2 Gastro-intestinal nematodes in cattle

1.2.1 Epidemiology

A wide variety of nematode species is found in the gastro-intestinal tract of farmed livestock in temperate regions (Agneessens et al., 2000; Borgsteede et al., 2000; Murphy et al., 2006). In cattle, two species are of particular importance, i.e. *Ostertagia ostertagi* and *Cooperia oncophora*. *O. ostertagi* is highly prevalent and highly pathogenic, while *C. oncophora* is less pathogenic but mainly important in concurrent infections. A clinical infection with these parasites results in gastroenteritis with diarrhoea, weight loss, anorexia and dehydration. Subclinical infections affect animal performance by impairing growth in young stock (Shaw et al., 1998b) and milk production in

adult cows (Charlier et al., 2009). The lifecycle of GIN is direct and consists of a parasitic phase and a free-living phase (Figure 1.1).

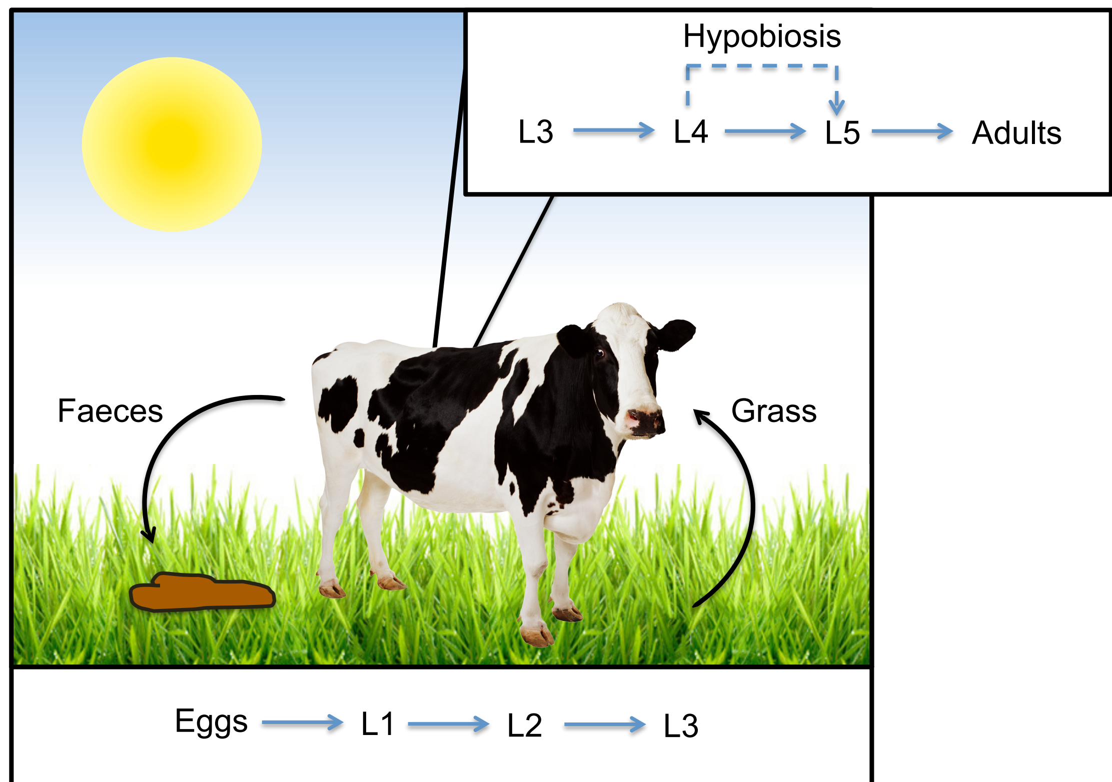

Figure 1.1 Parasitic and free-living phases of the lifecycle of gastro-intestinal nematodes (L1, L2, L3, L4 and L5 are respectively first, second, third, fourth and fifth stage larvae).

The parasitic phase of the gastro-intestinal nematode lifecycle

The parasitic phase commences when the host ingests infective third stage larvae (L_3) during grazing. After ingestion and arrival in the rumen, the infective L_3 cast their cuticle (exsheath), which they retained from the previous larval stage. The exact stimulus for this exsheathment is not known, but changes in pH, temperature and carbon dioxide concentration likely play a role along with the digestive secretions present in the rumen (DeRosa et al., 2008). Exsheathment is followed by migration of the L_3 to the predilection site, which depends on the nematode species (Table 1.1). After around 4 days post infection (p.i.), the larvae develop into L_4, which will develop to L_5 around 12 days p.i. When the adult stage is reached, the males and females mate and the females will subsequently produce eggs (Anderson, 2000). The prepatent period, which is the period between ingestion of L_3 to egg excretion,

differs slightly between nematode species but is on average 18 days (see also Chapter 3).

Table 1.1. Gastro-intestinal nematode species of cattle found in Belgium and the Netherlands (based on Agneessens et al., 2000 and Borgsteede et al., 2000).

Predilection site of adult worms	Nematode species
Abomasum	*Ostertagia ostertagi*
	Trichostrongylus axei
	Haemonchus placei
	Haemonchus contortus
Small intestine	*Cooperia oncophora*
	Cooperia punctata
	Trichostrongylus colubriformis
	Nematodirus helvetianus
	Bunostomum phlebotomum
	Capillaria bovis
Large intestine	*Oesophagostomum radiatum*
	Trichuris spp.

During the course of a prolonged infection with GINs, hosts generate acquired immunity, which affects the dynamics of the parasitic phase (Claerebout and Vercruysse, 2000). Subsequently a decrease in egg output and a stunted growth of the nematodes are seen, followed by a retarded development. Finally, adult worms are expelled and fewer ingested larvae establish. The immunity build-up is affected by factors related to both host and parasite. Host related factors that affect the rate and level of acquiring resistance to GINs are genetics, age, nutrition and farm management (van Houtert and Sykes, 1996; Shaw et al., 1998a; Kanobana et al., 2001). A parasite related factor that affects immunity development is the species of GIN considered. For example, immunity against *C. oncophora* is acquired more quickly and more strongly compared to *O. ostertagi* (Armour, 1989).

Under certain conditions, a proportion of the ingested larvae can cease their development and enter a period of arrested development, called

hypobiosis. The stage at which larvae become hypoactive and enter hypobiosis differs between nematode species. For *O. ostertagi*, *C. oncophora* and *Haemonchus* spp. the early L_4 can enter hypobiosis, while for *Trichostrongylus* spp. and *Nematodirus* spp. respectively the L_3 and late L_4 stages can arrest their development (Michel, 1974). A strong seasonality is seen in the occurrence of hypobiosis: in temperate climate regions, hypobiosis typically occurs in autumn and winter (Michel et al., 1974; Smith, 1979; Armour and Duncan, 1987), while in the Southern Hemisphere hypobiosis is mainly seen in spring (Smeal et al., 1980; Lutzelschwab et al., 2005). It is still not exactly understood what the driving factors of hypobiosis are, but both climate and immunity are described to have an important influence (Armour and Duncan, 1987; Eysker, 1993). Accordingly, variation in the ability to arrest within the same nematode species is described, indicating that genetic factors underlie the observed differences (Troell et al., 2006). Factors that influence the resumption of the maturation process are even less known. Some evidence supports that immunosuppressive conditions induce the maturation of arrested larvae (Michel 1971; Michel et al., 1979), however not all evidence supports this (Prichard et al., 1974; Gibbs, 1986). Others state that development resumes synchronously after a fixed period of 3 to 4 months (Armour and Bruce, 1974), but also theories of a steady turn-over to the adult population are described (Michel et al., 1976a; 1976b).

The free-living phase of the gastro-intestinal nematode lifecycle

The free-living phase starts with excretion of eggs produced by the female worms in the environment. Within the faecal pat these eggs hatch and develop into different larval stages. First, the L_1 stage hatches from the eggs. The L_1 moult into L_2, which consequently develop into the L_3 stage. Temperature is the main driver of both egg hatching and larval development, resulting in highly variable speeds for reaching the infective L_3 stage (Table 1.2). On pasture, the development from egg to L_3 may therefore take weeks in early spring compared to days during summer, while under winter conditions eggs will fail to develop into infective larvae (Rose, 1961; 1963). Besides temperature, the moisture level also affects the development of the free-living stages (Rossanigo and Gruner, 1995; O’Connor et al. 2006).

Table 1.2. Development rates of the free-living stages of *O. ostertagi* and *C. oncophora* for different temperature ranges under laboratory conditions (based on Rose, 1961; 1963).

Temperature (°C)	**Duration of development from egg to L3 (days)**	
	O. ostertagi	*C. oncophora*
10-11	18-28	21-56
14-16	7-16	4-21
22-23	3-7	3-9

To enable ingestion by grazing hosts, L_3 must escape from the faecal pat and migrate onto the surrounding herbage. Both passive and active movements of L_3 are described. Passive migration is important for the escape from the faecal pat and migration over greater distances. Rainfall is the main mechanism for passive migration of L_3, but transport by insects, earthworms, birds and cows is also described (Tod et al., 1971; Grønvold, 1979; 1984a; Hertzberg et al., 1992). During a rain shower, raindrops that land on the faecal pat can launch the larvae that are close to the surface. Most larvae are found in a radius of 30 cm around the pat, but passive dispersal due to rainfall of more than 90 cm has been recorded for *O. ostertagi* (Gronvold, 1984b; Gronvold and Høgh-Schmidt, 1989). Active migration of L_3 is limited to shorter distances, and therefore likely to be of main importance for movements towards the pat surface and between swards and soil (Rose, 1963; Silangwa and Todd 1964; Krecek and Murrell, 1988). Active movements of L_3 mostly depend on the level of humidity and to a lesser extent on temperature (Silangwa and Todd 1964). When relative humidity is high, significantly more larvae will show active migration compared to low levels of humidity (Wang et al., 2014). The impact of temperature on active L_3 movement is mainly important when moisture is not limited, with warmer temperatures stimulating larval movement.

Infective L_3 can survive for long periods on pasture. *O. ostertagi* and *C. oncophora*, for example, have been recovered from herbage for almost 2 years after deposition in the dung (Rose 1961; 1963). The L_1 and L_2 feed on

bacteria in the faecal pat, while the L_3 are not able to feed because they retain the cuticle of the L_2 stage. This extra cuticle serves as a protective sheath against detrimental environmental factors such as desiccation. However, because L_3 fall back on a limited non-renewable energy reserve, they are more vulnerable to high temperatures. In general, larval survival is prolonged by cool, dry weather and shortened by hot, wet weather (Barger, 1999). Additional factors that affect the survival of L_3 on pasture are the exposure to sunlight (i.e. UV-radiation) and presence of dung beetles (Fincher, 1975; van Dijk et al., 2009). There are two reservoirs for infective larvae: the faecal reservoir and the soil reservoir. The role of the faecal pat as a L_3 reservoir is primarily in periods of draught, during which typically a dry crust is formed on top of the cow pats that retains the larvae within the pat. Retention and survival of L_3 in faecal pats during long periods of droughts (18 months) is seen (Barger et al., 1984). A large proportion of the larvae that escape the faecal pat migrates towards the soil, which probably also acts as a shelter to facilitate their longevity (Alsaqur et al., 1982; Callinan and Westcott, 1986; Krecek and Murrell, 1988; Knapp-Lawitzke et al., 2014).

1.2.2 Control

Control of GINs is based on pasture management and/or treatment with anthelmintics. These anthelmintics belong to three distinct classes: benzimidazoles, imidazothiazoles and macrocyclic lactones. In previous decades, the control of GINs in cattle farming relied to a great extent on the intensive use of these products (Charlier et al., 2010). Several objections, however, can be made to the frequent application of anthelmintic products. For young stock, the preventive use of anthelmintics, for example, can prevent the development of an adequate immunity against GINs and lungworm infections (Vercruysse and Claerebout, 1997; Claerebout, 2002). Further, the presence of drug residues in food and the environment is a growing concern in the light of food safety and biodiversity (McKellar, 1997; Floate et al., 2005; Tsiboukis et al., 2010; 2013; Cooper et al., 2011). However, the increasing number of reports on anthelmintic resistance development is what really raised the alarm bells (Sutherland and Leathwick,

2011). Consequently, the search for more targeted and sustainable control strategies that secure the future use of the available anthelmintics while maintaining optimal animal performance, commenced (Charlier et al., 2014). The keystone of the currently proposed control approaches is maintaining a significant proportion of the parasite population unexposed to anthelmintics, in order to assure the propagation of susceptibility-associated genes to the next generation. This part of the worm population is found in untreated hosts and/or on pasture and is referred to as the population '*in refugia*' (van Wyk, 2001). Two new control approaches that are based on this concept are targeted treatments (TT) and targeted selective treatments (TST) (Kenyon and Jackson, 2012; Charlier et al., 2014). When applying TT, the whole herd is treated based on knowledge of the risk or severity of infection. When applying TST, only those animals in the herd that are thought to benefit the most from treatment based on indicators related to parasitological (e.g. faecal egg counts (FEC)), production parameters (e.g. weight gain, milk yield, body condition score (BCS)) or morbidity parameters (e.g. dag score, FAMACHA) are treated.

Besides the use of anthelmintics, non-chemotherapeutic control measures can be used to reduce the parasite infection pressure. Moreover, these non-chemotherapeutic control measures provide an extra support to minimise the use of anthelmintics, even when TT and TST would become standard practice in the future (Charlier et al., 2014). The non-chemotherapeutic control measure that is most within reach and hence most applied on farms at this moment, is pasture management (Charlier et al., 2010). In some regions, the implementation of certain measures is limited by the availability of grazable land. Mowing of pastures, postponing turn-out dates and assuring an age above 6 months before first turn-out, however, are feasible even when the amount of grazable land is limited. Other non-chemotherapeutic control approaches, such as vaccination and genetic selection, are only used in sheep farming. Recently, a vaccine against *Haemonchus contortus* based on purified native worm antigens has become commercially available (Bassetto et al., 2014). In Australia, New Zealand, South Africa and the U.K., genetic selection is used to breed sheep that are

more resistant and resilient to GIN infections (Woolaston and Baker, 1996; Bath, 2014).

1.3 Hosts and parasites in the 21st century

Over the past decades, several aspects concerning livestock, GINs and their host-parasite relation have drastically evolved compared to the situation of the 1960s, when the Food and Agriculture Organization of the United Nations (FAO) began keeping production records. This evolution is not brought to an end as many drastic changes are expected for the future half century. The host-parasite system is a tight network and the impacting factors will often interact resulting in a complex web of interrelated and sometimes opposing forces. Future control approaches thus need to be ‘holistic’, in which for each adaptation the consequences are considered before intervening (Gauly et al., 2013; Skuce et al., 2013). The following sections elaborate on the evolution of both hosts and parasites during the past half century, focuses on the expected trends to come and discusses the underlying drivers of the anticipated changes and their interactions.

1.3.1 Cattle in a changing world

In the previous century, the global livestock production has grown substantially by increasing the number of animals reared and, more importantly, by enhancing the productivity per animal (Thornton, 2010; Figure 1.2). In developed regions, cattle farms have disaggregated into specialised milk and beef industries that show 30% higher milk yields per animal and 30% higher carcass weights, respectively, compared to the 1960’s. The main drivers of these historical changes are genetics, nutrition and animal management. Genetic improvement by selection among and within breeds focusing on specific production traits has changed the composition of the modern bovine population (Lucy, 2001). The use of reproductive technologies such as artificial insemination, induction of superovulation and embryo transfer underpinned this genetic selection (Moore and Thatcher, 2006). Nutritional refinements and farm management improvements influencing

animal health and welfare have also had a positive impact on animal performance (LeBlanc et al., 2006). A strong informational need and collection of production records has become the cornerstone of modern livestock farming and is expected to become only more important in the future (Seidel, 2014).

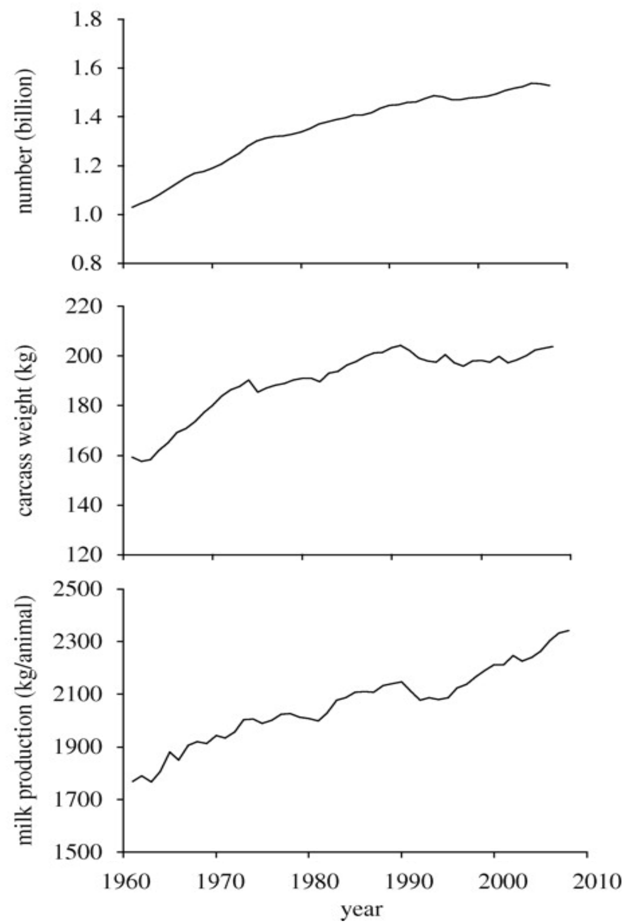

Figure 1.2. Global number of bovines (cattle and buffaloes), global carcass weight and global milk yields per animal from 1961 to 2008 (adapted from Thornton, 2010).

The toll of intensification

However, the high levels of animal performance reached today compromise other aspects of animal production and arguably animal welfare (Oltenacu and Broom, 2010). The gain in milk yield, for example, has lead to a decline in cow fertility, increased disease incidence and elevated stress sensitivity despite meeting nutritional and management requirements (Lucy, 2001; Hare et al., 2006; Norman et al., 2009). In developed countries, the

modern production systems themselves can be called more sustainable or environmentally friendly than the historical methods implemented back in 1940 because of their high efficiency level (Capper et al., 2009). However, the scale of growth and intensification that the industry has experienced takes a significant environmental toll locally and globally. It is common ground now that human activity, including agriculture and livestock production, is one of the primary causes of climate change (IPCC, 2013). Animal production is a significant source of green house gas emission that contributes to 18% of the total anthropogenic green house gas releases (Steinfeld et al., 2006). Ruminants emit methane following enteric fermentation and defecation, representing 35 to 40 % of the global anthropogenic methane emission (Steinfeld et al., 2006). Deposition of ammonia, phosphorus and nitrate originating from livestock manure to land or water is a serious concern for the longevity of natural ecosystems (Fangmeier et al., 1994; Kotak et al., 1993; Hansen et al., 2012; Shepherd and Newell-Price, 2013). Biodiversity is further threatened by expansion of grazing land and arable land for the production of feed crops (Alkemade et al., 2013). The environmental and ethical consequences of the increased productivity seen during the last decades have raised public concerns on the sustainability of the industry. Livestock production is increasingly questioned by concerns related to food safety and animal welfare (Boogaard et al., 2011a; 2011b; Croney and Anthony, 2011). It is therefore not a surprise that climate change, achieving ecological goals and raised public awareness are expected to be the main drivers that will shape future livestock production systems, together with providing food security. Global demand for livestock products is expected to double by 2050 (Steinfeld et al., 2006) and livestock industry will thus have to keep its key role in securing the world's food supply ('food security'), while operating against a background of increased climate variability and achieving stringent environmental and social goals.

Drivers of change: climate change

Climate change itself will act to magnify certain aspects of climate variability (IPCC, 2013). The frequency and intensity of extreme weather

events, such as heat waves, draughts and floods, is expected to increase in the future. Global average surface temperature is estimated to increase with 0.3 to 0.7°C by 2035. For Central Europe, in general, drier and hotter summers are expected (IPCC, 2013). The fact that animal production is affected by climate change while contributing to it at the same time, implicates a tight interplay of these two. The vulnerability of livestock to the effects of climate change depends on the geographical region and the implemented production system (e.g. grazing vs. non-grazing systems; Table 1.3) (Thornton, 2010; Godber and Wall, 2014; Sundström et al., 2014). The impact of climate change affecting livestock animals can be direct and indirect. Climate change will affect livestock directly by the occurrence of heat stress. High ambient temperatures and high levels of solar radiation and humidity that accompany heat waves, are stress factors that negatively impact animal health, animal welfare and feed intake. Although there is a significant risk for heat stress affecting animal production, the major effects are not expected to be encountered in Central Europe before 2050 (Skuce et al., 2013). The indirect effect of climate change on livestock animals is through affecting farm management practice and infectious disease dynamics. Pasture management is an important livestock management practice that can be affected by climate change in the future. The predicted increases in temperature will stimulate grass growth and as a consequence, farmers might be tempted to lengthen their cattle's grazing season (Skuce et al., 2013). Longer grazing seasons without increasing pasture size can compromise herbage quality and nutrient concentration constraining host physiology and immunity on their turn (van Houtert and Sykes, 1996; Gauly et al., 2013; Skuce et al., 2013). Disease incidence and distribution are also expected to change due to climate change, which will have an important impact on livestock production systems. This latter issue will be addressed in more detail, with a focus on parasitic diseases, further in this review (see 1.3.2).

Table 1.3. Impact of climate change on livestock production systems (adapted from Thornton, 2010).

	Grazing system	Non-grazing system
Direct	- Extreme weather events	- Extreme weather events
	- Droughts and floods	- Water availability
	- Productivity losses due to heat stress	
	- Water availability	
Indirect	- Fodder quantity and quality	- Increased resource price (e.g. feed, energy)
	- Host-pathogen interactions	- Increased cost of animal housing (e.g. cooling systems)
	- Disease epidemics	- Disease epidemics

Drivers of change: mitigation actions and ecological goals

In addition to climate change itself, other factors will be important drivers of change in future livestock systems. Imposed rules and legislative measures to achieve environmental goals and minimise the industry's contribution to climate change and farmer's attempts in mitigating the detrimental effects of climate change will play a role in how animal production will evolve the coming decades. A possible response to meet the increasing demand for food is 'sustainable intensification', which aims to increase agricultural production while minimising pressure on the environment (Fitzpatrick, 2013). The potential of the livestock industry in minimising its contribution to climate change lays mainly in decreasing the greenhouse gas emission by adapting farm practice and implementing new technologies (Gill et al., 2010). Potential routes to do this are by acting on emissions directly or by enhancing production efficiency and thus lowering the emissions per unit of food produced. Direct actions on emission include changing livestock diet composition and using methane or N_2O-inhibitors. The amount of methane eructated by ruminants, for example, can be affected by changing the energy

density of the diet (Yates et al., 2000), while the amount of nitrogenous components excreted in manure can be adjusted by changing tannin and/or protein levels in the feed (Paul et al., 1998; Misselbrook et al., 2005). Enhancing the efficiency of the production systems by intensification and adjusting manure and pasture management, is a proposed mitigation option that does not only limit green house gas emissions, but also cuts back the amount of land used for livestock production (Gill et al., 2010). In light of this, the implementation of zero-grazing systems might gain momentum. Improved efficiency can also be achieved, as proven in the past, by genetic selection. However, not only production traits, but also cow fertility and health will need to be taken into account to ensure sustainability and to achieve optimal efficiency (Gill et al., 2010; Buckley et al., 2014).

Drivers of change: public awareness and consumer's opinion

In affluent western countries, there is an increased public awareness concerning food production, and more specifically food production by the livestock industry. The complex issue of animal welfare, for example, gains more and more impact as it influences society's perception of the sector. Moreover, consumers begin to attribute certain product properties, such as food safety and food healthfulness, to animal welfare (Harper and Makatouni, 2002). Public opinion is capable of influencing modern management practices. For example, some might consider limited pasture access for dairy cattle as unacceptable for cow well-being (Boogaard et al., 2011b), which could counteract the current zero-grazing trend. Public messages promoting lowered meat consumption for ecological and health reasons might form an extra challenge for the future livestock sector (Hedenus et al., 2014; Stehfest et al., 2009; Popp et al., 2010). Likewise, it is possible that potential meat substitutes such as insects (entomophagy) and *in vitro* meat become more important in addressing the issue of food and feed security (Bonny et al., 2015; Bhat et al., 2015; Verbeke, 2015).

1.3.2 Gastro-intestinal nematodes in a changing world

Today, the key focus of health management in livestock production is disease prevention rather than treatment (LeBlanc et al., 2006). In the past decades, parasitic infections on cattle farms have also shifted from clinical cases resulting sometimes in death, to mainly subclinical cases limiting animal production. The introduction of highly effective anthelmintic substances in the 1980s together with improved parasitic disease monitoring and improved farm management have underpinned this evolution. In future decades, infectious disease patterns are expected to change but the impact of these changes is difficult to foresee. For nematode infections in sheep in the United Kingdom, some early evidence of the future trends is already provided and suggests that not only parasite abundance, but also seasonality and spatial distribution of GIN infections will be affected (van Dijk et al., 2008; Kenyon et al., 2009; McMahon et al., 2012). During the past few years, an increased number of cases of parasitic gastro-enteritis was observed. Moreover, a higher disease incidence later in the grazing season was seen and, specifically for *Haemonchus* spp., a higher prevalence was observed in the northern regions of the United Kingdom, suggesting a northwards spread for this species (van Dijk et al., 2008). The two important drivers of these observed trends are thought to be anthelmintic resistance and climate change. Interaction between these two drivers and with other factors that influence parasite epidemiology, such as farm management, make predicting future parasite disease patterns and designing adapted control strategies even more challenging.

Anthelmintic resistance

To date, the control of GIN infections on cattle farms is mainly based on the use of broad-spectrum anthelmintics, with macrocyclic lactones as the dominant class. However, resistance of parasite populations to all classes of anthelmintics has become widespread and is recognised as an increasing problem for cattle farming (Sutherland and Leathwick, 2011). Most reports on anthelmintic resistance in cattle concern limited efficacy of macrocyclic lactones against *Cooperia* spp., the dose-limiting parasite for this anthelmintic class. Confirmed cases of resistance against macrocyclic lactones for *O. ostertagi* remain rare (Edmonds et al., 2010). For a variety of cattle nematode

species resistance is also reported against the imidazothiazole derivatives and benzimidazoles (e.g. Eagleson and Bowie, 1986; Loveridge et al., 2003). Moreover, simultaneous resistance of nematode species to multiple anthelmintic classes in cattle has also been reported (Sutherland and Leathwick, 2011). This emerging phenomenon of anthelmintic resistance imposes the need for adapted control strategies that are effective in limiting production losses, while ensuring a maintained effectiveness of the available anthelmintics in the long term (see also 1.2.2). The keystone of the currently proposed control approaches is maintaining a significant proportion of the parasite population in *refugia* (van Wyk, 2001). Consequently, advice on worm control is shifting to treating selected individual animals rather than entire herds (Charlier et al., 2014).

Climate change and gastro-intestinal nematodes

Anthelmintic resistance, however, cannot alone account for the recently observed changes in parasitic disease patterns (van Dijk et al., 2008). Because climate is, together with farm management, one of the most important drivers of parasite epidemiology, the expected climate change scenarios will have an impact on parasite infection patterns. The effect of climate change on future parasite epidemiology is not as straight forward as early publications on this topic suggested (Kutz et al., 2005; Poulin, 2006) and interactions between climate change and anthelmintic resistance or farm management complicate forecasting even more (van Dijk et al., 2010). Climate change can have a direct and an indirect impact on the epidemiology of GIN infections.

Parasite abundance and larval availability are directly affected by climate through the influence of temperature and moisture on development, dispersion and mortality of the free-living stages. Future climate scenarios predict an increased daily temperature for temperate regions (IPCC, 2013), which theoretically can have opposite effects on the different parasite life stages. Higher temperatures will increase the development rate of eggs and early larval stages found in the faecal pat, but they will also increase the mortality of larval stages found on pasture, especially affecting larval survival during winter. The potential of the predicted temperature increase to affect

development or mortality, however, varies between different nematode species and, therefore, also the sensitivity of each nematode species to climate change varies (van Dijk et al., 2010). Moreover, not only does the threshold for development of the free-living stages differ between nematode species, but also species-specific needs exist for other life history traits, for example egg hatching in *Nematodirus battus* (van Dijk and Morgan, 2008; 2010). The moisture level is, certainly in temperate regions, not considered as a limiting factor for egg or larval development as this process occurs inside the cow pat (van Dijk and Morgan, 2012). However, rainfall impacts larval emergence from the faecal pat on to the herbage (Grønvold, 1984b; Grønvold and Høgh-Schmidt, 1989). Future predictions report long periods of drought followed by short periods of heavy rainfall, which could lead to sudden increases in larval emergence and pasture infectivity (van Dijk et al., 2010). The fact that an increased incidence of parasitic gastro-enteritis in sheep is observed over the past ten years, suggests that the forces stimulating larval availability overrule the ones that deter larval availability, at least for the United Kingdom (van Dijk et al., 2010; Rose et al., 2015). It is in fact a complex network of interactions that determines whether an increased parasite abundance also leads to an increased parasitic disease risk, as factors besides parasite population dynamics, such as host presence and host immunity, contribute to this (Kenyon et al., 2009; Skuce et al., 2013; Figure 1.3).

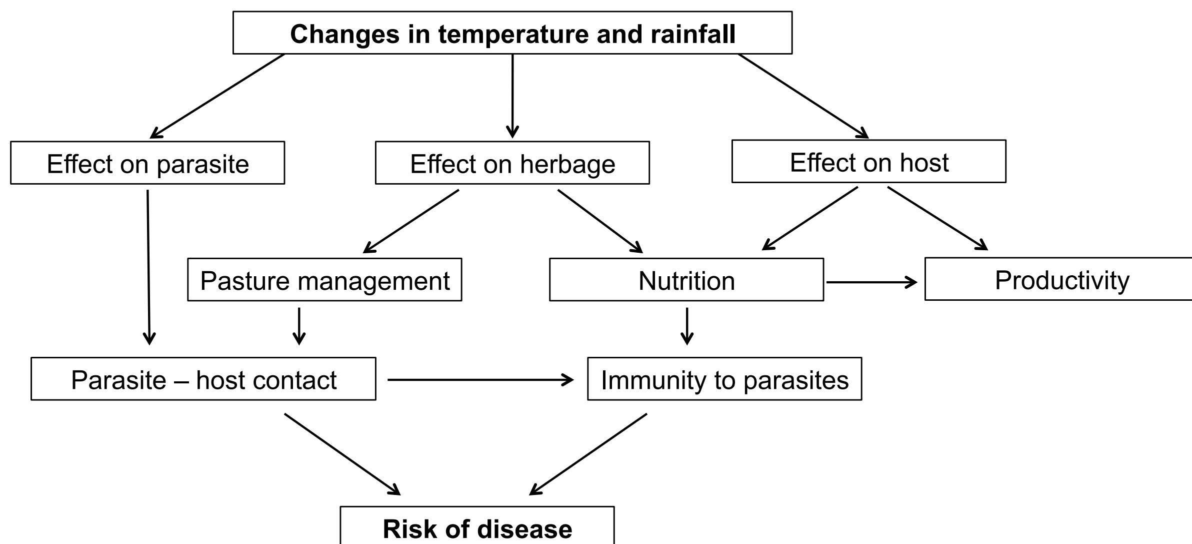

Figure 1.3. A simplified scheme of how climate change may affect the risk on parasitic disease (adapted from Kenyon et al., 2009).

Climate change can also indirectly influence parasite epidemiology by affecting farm management (see 1.3.1), by influencing the development of anthelmintic resistance or by influencing host immunity. If longer grazing seasons would be the future pasture management trend (Phelan et al., 2015), this means that GIN infections potentially have a larger window of opportunity and that the number of potential parasite generations per grazing season may be increased, probably increasing the overall pasture infection level (Skuce et al., 2013). Theoretically, this could lead to more frequent application of anthelmintic treatments and consequently the development of anthelmintic resistance could gain momentum (Morgan and van Dijk, 2012). Moreover, the detrimental effect of climate change on larval survival on pasture can diminish the *refugia* population, providing an extra push in the back for anthelmintic resistance development (Morgan and van Dijk, 2012). Climate change can compromise host immunity by negatively affecting the host's nutrition status (van Houtert and Sykes, 1996; Coop and Kyriazakis, 1999). Heat stress is for example associated with decreased feed intake (West, 2003) and grassland quality can be negatively influenced by the expected climate conditions (Gauly et al., 2013; see also 1.3.1). Luckily, also mitigation of these trends can be expected through certain anticipated adaptations and interventions (Morgan and Wall, 2009). For example, if the future implementation of zero-grazing systems in the dairy industry increases to enhance production efficiency and

decrease greenhouse gas emissions, the risk of pasture borne diseases such as GIN infections will be lowered. The use of zero-grazing systems can, however, negatively affect the incidence of other diseases (Haskell et al., 2006; Smits et al., 1992; Bruun et al., 2002; Burow et al., 2011), moreover, these animals will not have acquired sufficient immunity against GIN which becomes important when they are sold to farms that do pasture their animals (Skuce et al., 2013).

1.4 Gastrointestinal nematode transmission models: a tool to support sustainable parasite control

In general, a model can be described as a simplified representation of a complex phenomenon (Vynnycky and White, 2010). The term “model”, however, appears under several meanings in scientific literature and can refer to a tangible item such as an animal model, but also to something more conceptual as a mathematical or statistical model. In this thesis, it refers to mathematical models, and more specifically, to mathematical models that simulate the transmission dynamics of parasitic diseases. These ‘transmission models’ aim to provide a simplified and abstract illustration of the disease transmission process. In the field of veterinary parasitology, transmission models that simulate GIN infections have been around for several decades (e.g. Gordon et al., 1970; Gettinby et al. 1979; Smith and Grenfell, 1985; Ward 2006a; 2006b; Rose et al., 2015). Given the nature of parasite-host interactions, transmission models are important tools to represent and manipulate such complex processes and interactions (Scott and Smith, 1994). Forecasting, analysing, simulating and educating are the key aims that have driven the creation of transmission models that simulate GIN infections (Smith, 2011). As discussed above, parasitic disease patterns are expected to change the coming years, but the future look and impact of these changes is difficult to predict. Transmission models enable extrapolation of current knowledge to alternative scenarios and large temporal scales (Rose et al., 2015) and will therefore remain important to understand the impact of anthelmintic resistance and climate change on parasite epidemiology and to facilitate the implementation of sustainable control strategies.

There is a significant amount of literature devoted to the subject of modelling. For the uninitiated, however, the comprehension of this topic is hindered by the use of specific terms and model classifications. Moreover, the mixed use of terms between different fields of mathematical modelling adds to this confusion. The following section elaborates on key terms encountered in the field of parasitic disease modelling. Secondly, the development of these models is explained and an overview of the currently available models for GIN infections in ruminants (sheep and cattle) is given.

1.4.1 Terminology of mathematical models for infectious diseases: a dictionary of its own

Mechanistic versus empirical models

Most transmission models for GIN are 'mechanistic' models, which need to be distinguished from 'empirical' models (Fox et al., 2012). Empirical models are based on measurements and observations and are thus data-driven. They consider correlative relationships that are in line with the current understanding of the system of interest, but without fully describing the system's behaviour. A synonym sometimes applied for this class of models is the term correlative models (Fox et al., 2012). Mechanistic models on the other hand, are based on the current knowledge and understanding of the system of interest and can be referred to as process-oriented. They consider the mechanisms that underlie the system's behaviour and explicitly describe these. For infectious disease modelling in general, these models are typically compartmental and the population to be modelled is divided into compartments according to the subgroup they belong to (e.g. susceptible, infected, immune), which is visualised by flow diagrams (Vynnycky and White, 2010; Scott and Smith, 1994; Figure 1.4). In contrast to empirical models, mechanistic models are better placed to make predictions concerning parasite transmission and disease risk because extrapolation is less of a limitation (Fox et al., 2012). Mechanistic models, however, do require an in-depth understanding of the system to be modelled and make use of more inputs and parameters because they incorporate more biological detail (e.g. Rose et al., 2015). Lack of knowledge and adequate parameter estimates is therefore the

primary bottleneck encountered in the development of this kind of models (Fox et al., 2012). Consequently, the uncertainty dealt with in these models is in general higher than for empirical models. It needs to be noted that, in practice, the distinction between empirical and mechanistic models is not always that strict. Empirical models often do consider a limited understanding of the system to be modelled and most mechanistic models include and use some kind of empirical information (see further).

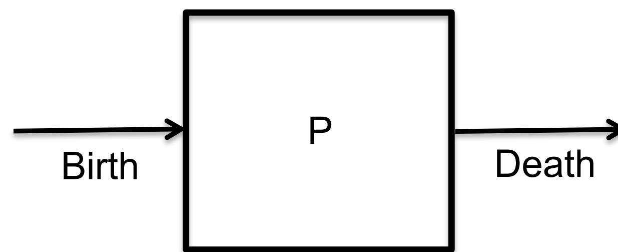

Figure 1.4. Example of a flow chart representing the population dynamics of a parasite population (box), with P the number of parasites in the population (adapted from Scott and Smith, 1994).

Macroparasites versus microparasites

For infectious disease modelling, a second distinction needs to be made between micro- and macroparasite models (Scott and Smith, 1994). Bacteria, viruses and protozoa are considered to be ‘microparasites’, as they are invisible for the naked eye and found in large, unquantifiable numbers in their hosts. Helminths (e.g. GIN) and arthropods are referred to as ‘macroparasites’, as they are much larger and present in a countable number in or on their hosts (Vynnycky and White, 2010). In general, macroparasites also require life stages outside the host for their propagation, while microparasites multiply solely within their host. Modelling these two broad classes of infectious agents requires in general a different approach (Zinsstag et al., 2015). For microparasites, all infected hosts can be considered equal. The prevalence is the subject that is actually modelled and the proportions of hosts belonging to the different disease categories (e.g. susceptible, infected, immune, recovered) are the state variables in such models. For macroparasites, however, the size of the infection matters and the models actually keep track of the mean number of different parasite life stages present in a host or the environment. These latter models can be referred to

as ‘burden models’, in contrast to the ‘prevalence models’ used for microparasites. The concept of the ‘basic reproduction rate’, R_0, is fundamental in describing and understanding the disease dynamics of microparasites. It is defined as ‘the expected number of secondary cases produced in a completely susceptible population by a typical infected individual during its entire period of infectiousness’ (Diekmann et al., 1990). For modelling macroparasites an analogous concept exists that is called the ‘basic reproduction quotient’, Q_0, which is defined as ‘the average number of offspring produced throughout the productive life-span of a mature parasite that survive to reproductive maturity in the absence of density-dependent constraints on population growth’ (Heesterbeek and Roberts, 1995). For both R_0 and Q_0 the same threshold value of 1 is applied. A value greater than 1 implies that the micro- or macroparasite can invade and maintain itself in the host population, while a value below 1 implies that the micro- or macroparasite population cannot persist (Heesterbeek and Roberts, 1995). In contrast with infectious disease models of microparasites, Q_0 is less widely used in macroparasite models.

Deterministic versus stochastic models

Another distinction is made between ‘deterministic’ and ‘stochastic’ models (Zinsstag et al., 2015; Vynnycky and White, 2010). A deterministic model assumes no variability or randomness and describes what happens on average in the system or process modelled. A stochastic model, however, incorporates the effect of chance events and the resulting random fluctuations in the population dynamics. Two types of stochasticity affect population growth, i.e. demographic and environmental stochasticity (Braumann, 2010). Demographic stocasiticity accounts for the variability in model variables arising from random differences among individuals, while environmental stochasticity takes the effect of environmental fluctuations on model variables into account (Braumann, 2010). A demographically stochastic model incorporates randomness and running the model multiple times with the same input will thus result in a distribution of variable outputs. Models that assume a homogeneous population, the so called ‘population-based models’, can be either deterministic or demographically stochastic (Zinsstag et al., 2015).

Models that assume a heterogeneous population, so called 'individual- or agent-based models', are in general considered to be demographically stochastic (Zinsstag et al., 2015; Vynnycky and White, 2010). In individual-based models, every individual of the population has its own characteristics and the model tracks the infection process for each of these individuals (Vynnycky and White, 2010).

1.4.2 Development of mathematical models for gastro-intestinal nematodes: a lifecycle of its own

The envisaged aim and intended application of the final model should be the main criteria in choosing the most appropriate model type. Mechanistic models allow extrapolation beyond the available knowledge and the observed temporal scales and are therefore better placed to forecast the impact of alternative climate conditions and control scenarios (Fox et al., 2012) and are therefore the further focus of this manuscript. The development of mechanistic models is a continuous process of structuring outline followed by parameterisation and validation, which in turn, is likely to be followed by fine-tuning outline and parameters and re-validation (Figure 1.5).

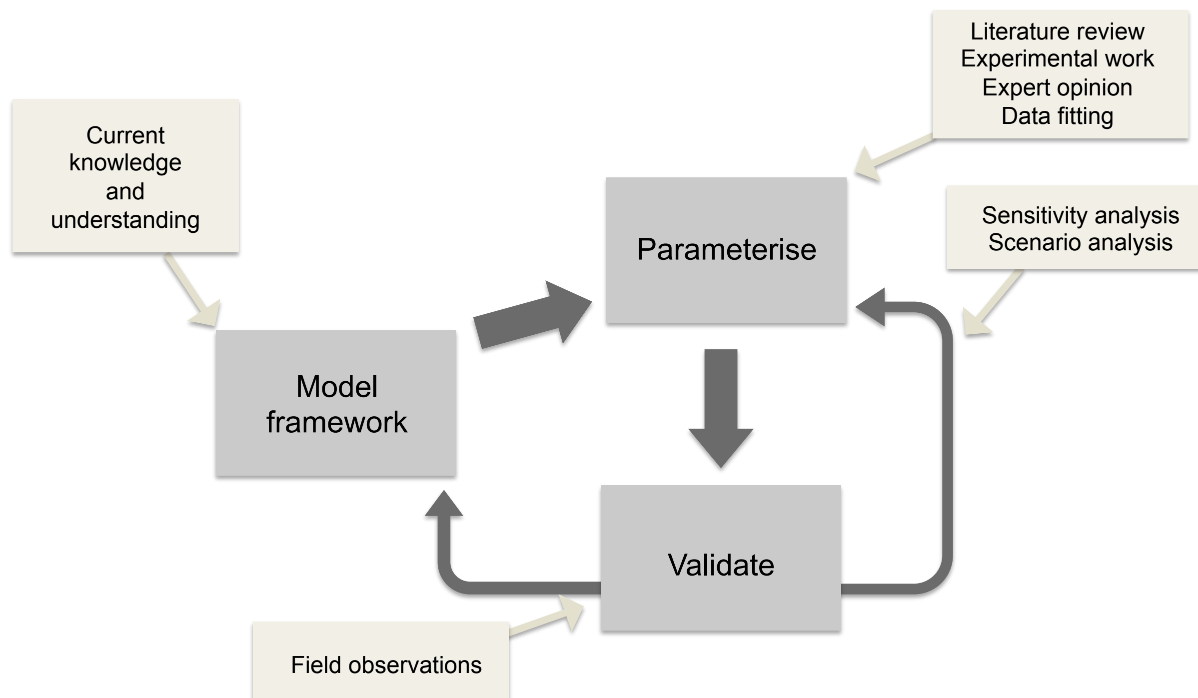

Figure 1.5. Overview of the different steps in the development process of mechanistic models for disease transmission.

Model structure

The first step in creating a mechanistic model is constructing the model's blueprint (Scott and Smith, 1994). This is typically pictured as a flow chart, in which the different model compartments (e.g. parasite life stages) are incorporated as separate entities that are connected. Most models for GIN are life cycle based models that simulate the different parasite life stages during both the parasitic and free-living phase. Decisions concerning the needed complexity of the model need to be driven by the envisaged goal, but the logic approach is to aim for a model that is as parsimonious as possible. A model only needs to be as detailed as required to provide useful insights into the research question that is investigated (Smith, 2011). The potential of creating highly complex models is constrained by the available level of understanding and the accessibility of adequate parameter estimates. Moreover, it needs to be noted that the usefulness of increased model complexity is constrained by the availability of adequate data for model input and validation (Morgan et al., 2004).

Parameterisation

Several sources can be consulted to obtain values to parameterise the model framework: literature review, experimental work, expert opinion and data fitting. If adequate data are available, a literature review is a logical start (e.g. Gaba et al., 2006b). Directly measuring life history traits (e.g. development time from egg to larvae) in laboratory experiments or field trials has the advantage that specific conditions can be created (e.g. van Dijk et al., 2009; van Dijk and Morgan, 2008; 2012). In some cases, however, parameter estimates cannot be obtained by measuring or observing and alternative methods need to be used. Consultation of experts in the considered field of research ('expert elicitation'), for example, is a tool to handle the lack of empirical data and obtain valid guesstimates (Refsgaard et al., 2006). A second alternative to obtain values for parameters that cannot be measured directly is fitting model predictions to real observations. The parameter value that provides the best fit between predictions and observations is then

implemented. This latter approach is an example of how the strict distinction between empirical and mechanistic models is not always justified.

Recently, an alternative approach for the parameterisation of models has found its introduction in the field of parasitology, namely fuzzy rule-based systems (Chaparro et al., 2010; 2011; 2013). Fuzzy logic allows the use of qualitative descriptions, when no other adequate data is available. It actually applies a human-like way of thinking in computer programming and allows the mathematical formulation of rather vague information. For example, a qualitative statement found in literature such as 'fewer L_3 were recovered during a period of drought' can be mathematically described and processed by the use of fuzzy rules. One would have to define 'a period of drought' and then generate if-then constructions based on this definition. If, in our example, drought occurs when there is no precipitation during one month, the construction becomes: IF monthly precipitation = 0, THEN L_3 mortality = 0.8, ELSE L_3 mortality = 0.1.

Uncertainty assessment

During the model development process uncertainty can arise from different sources and uncertainty assessment is needed during model creation. In general, three types of uncertainty must be accounted for: (1) methodological, (2) structural and (3) parameter uncertainty (Bilcke et al., 2011). Methodological uncertainty is caused by the lack of a normative approach for modelling certain aspects or to quantify certain parameters. Often, several acceptable methodological choices exist and modellers will need to make a rather subjective decision, as there is not always an intrinsically correct answer. Following a reference case as example or transparently reporting the choices and assumptions made are ways of dealing with methodological uncertainty and allowing others to follow the rationale of the modellers. Structural or model uncertainty arises from an incomplete knowledge on which features should be incorporated to fully grasp the disease dynamics and relevant drivers. Scenario analysis, in which different modelling approaches and/or assumptions are compared, can be applied to deal with both methodological and structural uncertainty (Bilcke et al., 2011). The

uncertainty derived from parameter measurement errors and lacking evidence or inability to estimate parameters, is referred to as parameter uncertainty. Moreover, the considered parameter estimates might not always be representative for the parasite species or region of interest. Sensitivity analysis and uncertainty analysis are ways to deal with this third kind of uncertainty (Bilcke et al., 2011). Sensitivity analysis attempts to identify key parameters by determining the change in model output that results from changes in model input, while uncertainty analysis describes the range of potential model outputs together with their associated probabilities of occurrence. Finally, uncertainty needs to be distinguished from variability. Where uncertainty mainly originates from a knowledge or information gap, random variation originates from the fact that populations are heterogeneous and that differences exist between and within individuals. Individual based models for example, aim to incorporate variation between individuals by taking specific characteristics for each individual of the population into account.

Model validation

Model validation is an important next step in the development process. Different aspects need to be considered when validating a mechanistic model and no absolute criteria exist. What exactly demonstrates a model's validity is a matter of discussion and is rather related to the intended applications and users of the model than to the model itself (Mayer and Butler, 1993). The model of Grenfell et al. (1987a), for example, was not validated against any observations. Later on, the authors raised the fact that 'a model is able to generate patterns that would be regarded as typical for a specific region by an experienced field worker', as a criterion for validity of GIN models (Smith and Grenfell, 1994; Smith, 2011). An objective assessment of such a criterion, however, seems to be difficult in practice and for models intended to extrapolate current knowledge to alternative scenarios in less known contexts, it beats the purpose. Nevertheless, model validation by comparison with field observations is not always straightforward and it is indeed unreasonable to expect precise correspondence between a single set of observations and

model output (Smith and Grenfell, 1994). Different approaches for objective assessment can be applied for model validation but no single approach is considered as the overall norm (Mayer and Butler, 1993). The display of observations together with simulations in time series plots, for example, aims to provide an overview of model performance in a rather intuitive manner, but can pose difficulties for exact interpretation (Mayer and butler, 1993) or can even be misleading (Smith, 2011). Regression analysis of observations versus simulations is therefore a great added value in model validation (Mayer and butler, 1993). An additional inconvenience of validating mechanistic GIN models with field observations is that it requires data with specific characteristics and a high level of detail demanding intensive data collection. These kinds of data are often not readily available. Although most papers don’t report it, the model development process is in general not finished after validation of the first model version. Going back to the drawing board and adjusting model structure and/or model parameterisation will highly likely follow the first validation.

Model development is in fact a cyclic process. Reporting on model development in a transparent way, including uncertainty assessment and model validation, is important to facilitate further research, but was often neglected in the past. The final step in the development process is the application and practical implementation of the model, which can be an elaborate research project on its own.

1.4.3 Mathematical transmission models for gastro-intestinal nematodes in farmed ruminants: an overview

The first transmission models that describe GIN infections in ruminants were developed during the mid-1960s. Modellers often described their model in several subsequent papers or performed follow-up research by extending existing model frameworks. Several reviews elaborate on the description of these models and the challenges faced (e.g. Smith and Grenfell, 1994; Bishop and Stear, 2003; Cornell, 2005; Smith, 2011; Fox et al., 2012), but an easy-to-access and comprehensible overview of existing models is lacking. Even though classifying these models in distinct categories is not always

straightforward and possible, Table 1.4 aims to provide a comprehensive overview of the available mechanistic models to help future model developers. We did not only consider GIN models of cattle, but also included those of sheep and farmed ruminants in general, because analogies can be drawn in their development and application. Following Smith and Grenfell (1994), the models were labelled as either generic or specific. Generic models provide a framework that aims to assess general dynamics of parasite infections. They rather consider a group of similar parasites (i.e. GIN) instead of specific parasite species. In general, they do not incorporate excessive amounts of biological detail and their structure is kept rather simple to not obscure key processes. Specific models describe the population dynamics of a particular parasite species and sometimes of a specific region or specific management situations. They often contain a greater deal of biological detail compared to their generic variants. A logic approach would have been to first develop generic models, which then provide a firm base to underpin the further development of specific models for distinct nematode species and/or research aims. However, this has not been the general approach of model development for GIN in ruminants (Smith, 2011). Authors often described their model in several subsequent papers and therefore an attempt was made to bundle joint papers as much as possible in Table 1.4. Certain models were further developed in follow-up research by extending the framework or sometimes several existing models were combined into one model. A strict distinction between stochastic and deterministic models is not always clear since some deterministic models include some effects of stochasticity (Cornell, 2005), for example by accounting for heterogeneity in the host or parasite population (e.g. Roberts and Heesterbeek, 1995; Barnes and Dobson, 1990a; 1990b). The largest number of available models is found to consider GIN infections in sheep, while fewer models are available for cattle or ruminants in general. The models for cattle even focused on only one nematode species, namely *O. ostertagi*. There are several fields of application that exist and according to Smith (2011) models of parasitic diseases in farmed ruminants fall into two distinct categories: predictive and illustrative models. Predictive models aim to forecast the occurrence and severity of disease, while illustrative models serve the aim of simulation, analysis and

education. The latter are for example used to improve the understanding of the impact of applying different control approaches on the infection levels or the development of anthelmintic resistance. The actual categorisation of the existing models in one of these two categories (predictive versus illustrative) is rather arbitrary and not feasible in practice, but the fact remains that even after all these years of research models are mainly successful when used for illustrative purposes rather than predictive purposes (Cornell, 2005; Smith, 2011). The large variety of influencing factors and features present in the parasite-host system make providing reliable predictions a lot more complex for macroparasites compared to microparasites (Cornell, 2005). Important features inherent to the dynamics of GIN infections that have challenged modellers are incorporating the effects of heterogeneity, parasite aggregation and acquired immunity.

Dealing with heterogeneity

An important technical improvement was the large increase in computational ability (Cornell, 2005). As a result of this increasing computer power, the number of models incorporating stochasticity has increased over the years. Along with this trend, an increasing number of individual-based models have been published the last few years. However, for models that consider GIN in cattle, no stochastic or individual based variants exist (Table 1.4). The advantage of these models is that they explicitly recognise the stochastic nature of the infection dynamics and deal with variation and differences in the host population. They are therefore able to capture a large range of phenomena. The complexity of such models, however, can sometimes impair the theoretical understanding while mean-field models provide a much more tractable solution.

Dealing with aggregation

The distribution of parasites in a host population is typically aggregated, which means that the largest proportion of the parasites are found in only a relatively small proportion of the available hosts (Shaw and Dobson, 1995). The negative binomial distribution is commonly used to describe these aggregated parasite populations and the level of aggregation can be expressed by a parameter, k, which is negatively correlated with the

aggregation level. However, none of the models for GIN in farmed ruminants, besides one (Cornell et al., 2004), incorporated the effect of parasite aggregation. Smith and Guerrero (1993) raised the question whether it is actually needed to incorporate parasite frequency distribution in mechanistic models and whether ignoring parasite frequency distribution would compromise model performance. Based on sensitivity analysis, they showed that model predictions did not differ much whether a model incorporated aggregation or not on the condition that the level of k is greater than 1. They also showed that for *O. ostertagi* and *C. oncophora* infections in calves the estimated value of k is in general greater than 1, which is typical for populations with high population means (Smith and Guerrero,1993).

Dealing with acquired immunity

Incorporating acquired immunity and its impact on parasite population dynamics during the parasitic phase is seen by many modellers as a difficult challenge (Cornell, 2005). The early models applied two different schools of thoughts to model immunity, i.e. the threshold hypothesis versus the turnover hypothesis (Smith, 1994). Following the threshold hypothesis, a threshold level of antigenic stimulation under the form of adult worms needs to be exceeded before a substantial host immune response is generated (Dineen et al., 1965). The turnover hypothesis assumes a more continual process of accumulation and loss of adult worms (Michel, 1963). The two hypotheses, however, originated from observations on two different nematode species (*H. contortus* and *O. ostertagi* for the threshold and turnover theory, respectively) and the distinction between the two theories was not always clear. The currently accepted approach is to assume that a host's immune 'status' increases with exposure to infective L_3 and wanes during periods in which the infection rate is low (e.g. during housing) (Roberts and Grenfell, 1991). The lack of direct observations of immunological processes and the inability to directly quantify the level of acquired immunity against GIN make it difficult to determine the appropriate mathematical incorporation and parameterisation in models.

1.5 Conclusion

In conclusion, the major challenge of the coming years for the cattle industry will be to ensure food availability and safety in a way that is ethically and environmentally acceptable while maintaining economic viability. To maintain or even increase future production levels, the control of GIN will remain important, but is challenged by the need to decrease the use of anthelmintic products while increased climate variability affects parasite epidemiology. The existence of interventions that mitigate climate change and increase productivity at the same time, provide important opportunities for the future. Tools that underpin an approach that takes the consequences of each intervention into consideration can support the orchestration of the complex interplay of influencing factors. Mathematical models can serve their purpose here as they enhance our understanding of how the epidemiological pattern of GIN infections will be affected by changes in its main drivers. A large amount of research is devoted to the subject of modelling GIN in farmed ruminants, but the lion's share of models deal with GIN species in sheep. Several applications of these models exist, but they are most applied for illustrative purposes (analysis, simulation, education) and not to make on-farm predictions. Often encountered bottlenecks in the development of mechanistic models for GIN are the lack of purpose driven data and the fact that acquired immunity is only partially understood.

Table 1.4. Overview of different mechanistic models for gastro-intestinal nematode (GIN) infections in cattle and sheep.

Reference	Original model (yes/no)	Expansion or application of an original model	Host species	Generic/ specific	Parasite species	Lifecycle stage	Stochastic or deterministic	Individual based model (yes/no)	Validated against field data
Tallis and Leyton, 1966; 1969	Yes	-	Ruminants	Generic	-	Entire life cycle	Stochastic	No	No
Roberts and Grenfell, 1991; Roberts and Grenfell, 1992	Yes	-	Ruminants	Generic	-	Entire life cycle	Deterministic	No	No
Roberts and Heesterbeek, 1995	No	Expansion of Roberts and Grenfell, 1991; 1992.	Ruminants	Generic	-	Entire life cycle	Deterministic	No	No
Marion et al., 1998; 2000	No	Expansion and stochastic reformulation of Roberts and Grenfell, 1991	Ruminants	Generic	-	Entire life cycle	Stochastic	No	No
Cornell et al., 2004	Yes	-	Ruminants	Generic	-	Entire life cycle	Stochastic	No	No
Rose et al., 2015	Yes	-	Ruminants	Generic	-	Free-living phase	Deterministic	No	No
Gettinby et al., 1979; Gettinby and Paton, 1981	Yes	-	Cattle	Specific	*O. ostertagi*	Entire life cycle	Deterministic	No	Yes
Smith and Grenfell, 1985; Grenfell et al., 1987a; 1987b; Smith et al., 1987a	Yes	-	Cattle	Specific	*O. ostertagi*	Entire life cycle	Deterministic	No	No
Smith et al., 1987b	No	Application of Grenfell et al., 1987a	Cattle	Specific	*O. ostertagi*	Entire life cycle	Deterministic	No	No

Ward, 2006a; 2006b	No	Extension of Grenfell et al., 1987b	Cattle	Specific	*O. ostertagi*	Entire life cycle	Deterministic	No	Yes
Chaparro et al., 2010	Yes	-	Cattle	Specific	*O. ostertagi*	Free-living phase (Develoment from egg to L3)	Fuzzy rule-based system	No	Yes
Chaparro et al., 2011	Yes	-	Cattle	Specific	*O. ostertagi*	Free-living phase	Fuzzy rule-based system	No	Yes
Chaparro et al., 2013	Yes	-	Cattle	Specific	*O. ostertagi*	Parasitic phase	Fuzzy rule-based system	No	No
Tallis and Donald, 1964; 1970	Yes	-	Sheep	Generic	-	Free-living phase (Distribution of L3 on pasture)	Deterministic	No	No
Callinan et al., 1982	Yes	-	Sheep	Generic	-	Entire life cycle	Deterministic	No	Yes
Leathwick et al., 1992; 1995	Yes	-	Sheep	Generic	-	Entire life cycle	Deterministic	No	
Bishop and Stear, 1997	Yes	-	Sheep	Generic	-	Entire life cycle	Stochastic	Yes	No
Roberts and Heesterbeek, 1998	No	Application and extension of Roberts and Heesterbeek, 1995	Sheep	Generic	-	Entire life cycle	Deterministic	No	No
Louie et al., 2005; 2007	No	Extension of Roberts and Grenfell, 1991; 1992	Sheep	Generic	-	Entire life cycle	Stochastic	Yes	No
Vagenas et al., 2007a; 2007b	No	Based on Louie et al., 2005	Sheep	Generic	-	Parasitic stage	Deterministic	No	No
Vagenas et al., 2007c	No	Extension and application of Vagenas 2007a; 2007b	Sheep	Generic	-	Parasitic stage	Stochastic	No	No

Leathwick et al., 2008; Leathwick 2012; Leathwick and Hosking, 2009	No	Applications of Leathwick et al., 1992; 1995.	Sheep	Generic	-	Entire life cycle	Deterministic	No	No
Fox et al., 2013	No	Expansion of Roberts and Grenfell, 1991 by combining it with Marion et al., 2005	Sheep	Generic	-	Entire life cycle	Stochastic	Yes	No
Gomez-Corral and Garcia, 2014	Yes	-	Sheep	Generic	-	Entire life cycle	Stochastic	No	No
Gordon et al., 1970	Yes	-	Sheep	Specific	*H. contortus*	Entire life cycle	Deterministic	No	No
Paton and Gettinby, 1983	Yes	-	Sheep	Specific	*T. circumcincta*	Entire life cycle	Stochastic	No	Yes
Paton et al., 1984	Yes	-	Sheep	Specific	*T. circumcincta*	Entire life cycle	Deterministic	No	Yes
Gettinby et al., 1989; Gettinby 1989	No	Expansion of Paton et al., 1984	Sheep	Specific	*T. circumcincta*	Entire life cycle	Deterministic	No	No
Dobson et al., 1990	Yes	-	Sheep	Specific	*T. colubriformis*	Parasitic phase	Deterministic	No	No
Barnes and Dobson, 1990a; 1990b; Barnes et al., 1988	Yes	-	Sheep	Specific	*T. colubriformis*	Entire life cycle	Deterministic	No	Yes
Echevarria et al., 1993	No	Adaptation of Gettinby et al., 1989	Sheep	Specific	*H. contortus*	Entire life cycle	Deterministic	No	No
Barnes et al., 1995; Dobson et al., 1996	No	Application and expansion Dobson et al., 1990a	Sheep	Specific	*T. colubriformis*	Parasitic phase	Deterministic	No	No
Kao et al., 2000	No	Application of Roberts and Heesterbeek, 1995	Sheep	Specific	*Teladorsagia spp., Trichostrongylus spp., H. contortus*	Entire life cycle	Deterministic	No	No

Learmount et al., 2006	Yes	-	Sheep	Specific	*Teladorsagia spp., Trichostrongylus spp., Haemonchus spp.*	Entire life cycle	Deterministic	No	Yes
Gaba et al., 2006a	Yes	-	Sheep	Specific	*T. circumcincta*	Entire life cycle	Stochastic	Yes	No
Guthrie et al., 2010; Learmount et al., 2012	No	Applications of Learmount et al., 2006	Sheep	Specific	*Teladorsagia spp., Trichostrongylus spp., Haemonchus spp.*	Entire life cycle	Deterministic	No	Yes
Gaba et al., 2010; Gaba et al., 2012	No	Application and expansion Gaba et al., 2006	Sheep	Specific	*T. circumcincta*	Entire life cycle	Stochastic	Yes	No
Laurenson et al., 2011	No	Expansion of Vagenas et al., 2007a; 2007b	Sheep	Specific	*T. circumcincta*	Entire life cycle	Deterministic	No	No
Singleton et al., 2011	No	Expansion of Bishop and Stear, 1997	Sheep	Specific	*T. circumcincta*	Entire life cycle	Deterministic	No	No
Dobson et al., 2011a; 2011b	No	Expansion and application of Barnes and Dobson, 1990a	Sheep	Specific	*T. circumcincta, T. colubriformis, H. contortus*	Entire life cycle	Deterministic	No	Yes
Laurenson et al., 2012a; 2012b; 2013	No	Applications of Laurenson et al., 2011	Sheep	Specific	*T. circumcincta*	Entire life cycle	Deterministic	No	No
Leathwick, 2013	Yes	-	Sheep	Specific	*T. circumcincta, T. colubriformis, H. contortus*	Free-living phase (Development from egg to L3)	Deterministic	No	No
Prada Jiménez de Cisneros et al., 2014	No	Extension of Singleton et al., 2011	Sheep	Specific	*T. circumcincta*	Entire life cycle	Stochastic	Yes	No

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OBJECTIVES

In farmed ruminants, infections with gastrointestinal nematodes (GINs) have an important impact on animal performance. Both climate and farm management are significant drivers of these parasites' epidemiology. Climate change and anthelmintic resistance are expected to result in an altered parasite epidemiology and constitute a strong call for alternative control approaches. Mathematical models that simulate the dynamics of GIN infections have great potential to provide improved understanding of parasite epidemiology under altered conditions and to support the development of alternative parasite control strategies. The majority of the existing models, however, have been developed for GIN infections in sheep. Moreover, models considering cattle have focused on only one GIN species, i.e. *O. ostertagi*. The lack of suitable process-oriented data is still one of the biggest challenges for modelling parasitic lifecycles and parameterisation of the existing mechanistic models is based on data of only a limited number of experiments. Finally, an extensive validation of mechanistic models for GIN infections based on sufficient field observations is limited to date.

The overall objective of this PhD research project was to develop a generic framework for a mechanistic transmission model that simulates the parasitic phase of the GIN lifecycle in farmed ruminants. Further, facilitation of the collection of pasture larval count data, a key input parameter, was explored. This PhD research project is part of collaboration between the Laboratory for Parasitology (Ghent University) and the Schools of Biological and Veterinary Sciences (University of Bristol). A complementary model that simulates the free-living phase of the lifecycle was developed by researchers at the University of Bristol (Rose et al., 2015).

The specific research aims were:

1. To quantify the main life history traits of the parasitic phase for *O. ostertagi* and *C. oncophora* through systematic review and meta-analysis and to assess the potential effect of immunity on these traits (**Chapter 2**).

2. To develop a conceptual framework for a mechanistic transmission model of the parasitic phase of GIN in farmed ruminants **(Chapter 3).**

3. To parameterise and validate this model for *O. ostertagi* and *C. oncophora* using field data (**Chapter 3**).

4. To facilitate the estimation of a key input parameter for transmission models of GIN, i.e. pasture larval contamination, by comparing two different sampling methods (**Chapter 4**).

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CHAPTER 2

QUANTIFICATION OF THE PARASITIC PHASE: SYSTEMATIC REVIEW AND META-ANALYSIS

PART A. QUANTIFICATION OF THE PARASITIC PHASE FOR *O. OSTERTAGI*.

Adapted from

Verschave S.H., Vercruysse J., Claerebout E., Rose H., Morgan E.R., Charlier J., 2014. The parasitic phase of Ostertagia ostertagi: quantification of the main life history traits through systematic review and meta-analysis. International Journal for Parasitology 44, 1091-1104.

2A.1 Introduction

O. ostertagi is one of the most prevalent GI roundworms of cattle in temperate regions with major constraints on productivity (Charlier et al., 2009). Because climate and farm management drive this parasite's epidemiology, global change affecting both factors could increase challenges to the future control of *O. ostertagi* (Morgan et al., 2013). Predictions of climate change including increasing ambient temperature in temperate regions are expected to affect parasite development and survival resulting in altered infection levels (Semenza and Menne, 2009; van Dijk et al., 2010; Molnár et al., 2013). In addition, intensification of modern dairy farming leads to a wide range of management alterations relating to nutrition, housing and grazing patterns (Herrero and Thornton, 2013) that may result in changes to infection pressure and seasonal patterns of exposure of livestock to infective stages. Besides these changes that affect parasite transmission and epidemiology, increasing reports of anthelmintic resistance strengthen the need for alternative and innovative parasite control approaches (Kenyon and Jackson, 2012; Höglund et al., 2013).

Mathematical models of infectious diseases have great potential to provide improved understanding of disease epidemiology and factors affecting it (Altizer et al., 2006; Woolhouse, 2011). Also in nematode control of ruminants such models are increasingly applied with the ultimate goal to support the development of practical parasite control strategies (Smith et al., 1987a; Learmount et al., 2006; Ward, 2006a, 2006b; Grassly and Fraser, 2008; Chaparro and Canziani, 2010). In the past, several mechanistic models based on the life cycle of *O. ostertagi* were developed (Gettinby et al. 1979; Gettinby and Paton, 1981; Smith and Grenfell, 1985; Grenfell et al., 1987a; 1987b; Smith et al., 1987a).

A major limitation on mechanistic models of parasite life cycles is parameter estimation. Where parameter uncertainty is significant, models frequently make use of expert knowledge, or scaling parameters derived from fits of predicted outputs with observed data (e.g. Dobson et al., 2011; Chaparro et al., 2013). While expedient, these approaches are vulnerable to changing conditions, such that the relationships that underpinned inference

under specific conditions no longer hold in different places or times, including under future global change scenarios. There is therefore a strong and ongoing need for thorough, transparent and unbiased estimation of key life history parameters, in order to properly inform models of parasite dynamics. Explicit reporting of uncertainty around such estimates is also valuable to guide and prioritise future experiments. While systematic review and meta-analysis are core methods in biological, medical and veterinary sciences, and are gaining increasing traction in a wide range of subjects (Lean et al., 2009), their use has been limited to date in estimating parameters for parasite transmission models.

Parasite density during the parasitic phase of *O. ostertagi* and the egg output is a function of four main life history traits: (1) larval establishment, (2) hypobiosis, (3) adult mortality and (4) female fecundity (Figure 2.1). Because acquired immunity is known to modulate these traits (Claerebout and Vercruysse, 2000), factors related to immunity development such as duration of exposure, intensity of infection and host age, need to be taken into account when quantifying life history traits of the parasitic phase. Former transmission models of *O. ostertagi* used trait estimates that were based on a limited number of experiments, to parameterize the parasitic phase (e.g. Anderson and Michel, 1977; Gettinby et al. 1979; Smith and Grenfell, 1985). During recent decades many infection trials with *O. ostertagi* were performed for various purposes (e.g. drug efficacy trials, host-parasite interaction studies). These studies enable us to make new estimates based on a larger number of experiments. Future transmission models will benefit from more accurate estimates of these parameters and their variation, but so far no attempt has been made to collect and summarize the available literature.

The aim of this study was to (1) quantify the main life history traits of the parasitic phase of *O. ostertagi* and (2) assess potential influences associated with the effect of immunity on these traits. A systematic review and meta-analysis was performed covering studies from 1962 to 2007 in which helminth-naïve cattle were artificially infected with *O. ostertagi.*

2A.2 Materials and methods

2A.2.1 Parameter definition

The four main life history traits of the parasitic phase of *O. ostertagi* addressed in this study are (1) the larval establishment rate, (2) the hypobiosis rate, (3) adult mortality and (4) female fecundity (Figure 2.1). Table 2.1 provides the definitions for these traits as used in this study.

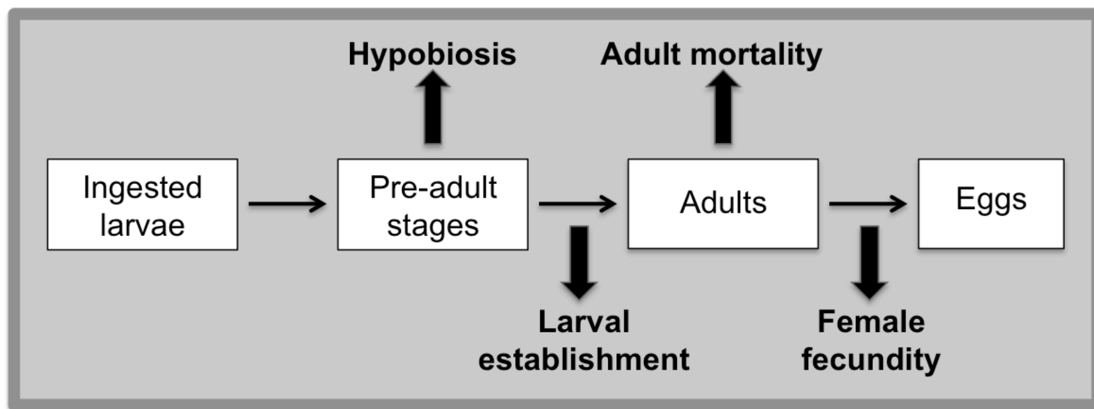

Figure 2.1 Schematic overview of the parasitic phase of *O. ostertagi* and its main life history traits.

2A.2.2 Search strategy and eligibility criteria

Peer-reviewed publications were the major source for data collection. In addition, data from former unpublished studies carried out at the Laboratory of Parasitology (Ghent University) were collected and added to the database. These latter data were generated in the context of *O. ostertagi* vaccination or anthelmintic efficacy research. They are further referred to as 'UGent trial (1-7)'.

For the systematic review, the electronic database ISI Web of Science was explored using the following general keywords: *(cattle OR bovine OR cow OR heifer OR bull OR steer OR calf OR calves) AND (nematode OR helminth OR parasit* OR trichostrongyl* OR ostertag* OR Cooperia OR oncophora) AND (infect* OR transm*).* No restrictions were placed on publication year or language. The search was performed until items published on or before February 6, 2012. The obtained literature was first subjected to a title based selection, followed by a second selection based on the reading of

the full text. All studies, in which naïve bovines were artificially infected with *O. ostertagi* using single or trickle infection protocols, were considered eligible on the conditions that (1) no anthelmintic treatment was applied during the infection and (2) they reported abomasal worm counts (individual or aggregated) after necropsy with an associated measure of variance (*i.e.* standard deviation, standard error of the mean).

To extract data for the quantitative analysis, specific eligibility criteria were used for each life history trait. For the calculation of larval establishment and hypobiosis only studies in which the duration of infection (*i.e.* time period between first infection and necropsy) was a minimum of 21 days were considered, because this is considered as the average pre-patent period of *O. ostertagi* (Anderson, 2000). In the establishment database a second eligibility criterion that only allowed studies with a duration of infection of less than 40 days was applied to reduce confounding due to adult mortality. Similarly, for the calculation of the adult mortality only studies with a minimal duration of infection of 40 days were used. To estimate hypobiosis, studies that distinctively reported counts of early L_4 stages (individual or aggregated) with the associated measure of variance were needed. In addition, to ensure that counted early L_4 were in fact arrested larvae, only studies with a time lag between last infection and necropsy of at least two weeks were used, to allow for maturation of non-arrested L_4 (Anderson, 2000). Female fecundity estimates required faecal egg count data (individual or aggregated) close to the moment of necropsy with an associated measure of variance. Based on the morphology of the eggs, no differentiation can be made between the GIN species considered. Therefore, experiments in which animals received concomitant infections with GIN other than *O. ostertagi* were excluded from the database of female fecundity. Finally, the average proportion of females present in the abomasum of an infected animal was also needed to estimate female fecundity. Since no summary data on the sex ratio of *O. ostertagi* are available in literature, studies were selected in which female and male worm numbers were reported separately for each experimental animal used.

2A.2.3 Data extraction and 'effect measure' calculation

From all eligible studies relevant data, including general study descriptions (*i.e.* year, study location, sample size), details on experimental animals (*i.e.* age, breed, gender, body weight) and information on study characteristics (*i.e.* infection mode, infection dose, *O. ostertagi* isolate source, concomitant infections with other nematode species) were extracted. If a publication contained more than one group of animals studied, then these were considered as different experiments. Finally, four separate spreadsheets (Excel, Microsoft Corp., Redmond, WA, USA) were created containing the extracted data for the quantification of the four different life history traits of *O. ostertagi*. Additionally, a fifth spreadsheet was created containing individual animal data suited to calculate the average proportion of female worms.

In each of the corresponding spreadsheets, the life history traits and their variance were calculated as effect measure per experiment based on the equations in Table 2.1. If needed, individual worm or egg counts were converted to aggregated values (arithmetic mean and variance) first. To calculate female fecundity, an estimate of the daily faeces production (DFP) was needed. First the daily manure production was computed based on the formula of Nennich et al. (2005) using average animal bodyweights reported for each experiment. If no data on bodyweight were available, standard age related growth curves for dairy cattle were used (Cue et al., 2012). Next, the average proportion of urine found in cattle manure was estimated based on data provided in Nennich et al. (2005) and Massé et al. (2014). The DFP was finally calculated by correcting the estimated amount of manure with the average proportion of urine found in cattle manure. The average proportion of female worms (F) was calculated based on the created dataset by dividing numbers of females found, by the total number of adult worms in each experimental animal.

2A.2.4 Meta-analysis

Based on the calculated effect measures, an inverse variance weighted average was computed for each life history trait by using a random-effects analysis. In order to explain the heterogeneity between experiments, several

moderator variables were evaluated in a mixed-effects analysis: infection protocol (single or trickle infection), infection dose (total number of L_3 given per animal), duration of infection (days), host age (days) and whether or not animals received concomitant infections with other nematode species besides *O. ostertagi* (mixed or mono infection). To provide a better fit of the model to the data, stepwise least squares polynomial fits and logarithmic transformations of moderator variables were performed. Full and reduced models were compared via likelihood ratio tests. The average proportion of female worms present in the abomasum was estimated based on individual animal data using a random-effects analysis with study reference as random effect.

Reported *P*-values in the text are those derived from univariate analysis, while *P*-values in table 6 are those derived from the final model containing significant moderators. If significant correlation existed between moderators, the less biological relevant was left out of the final model. All analyses were performed in R version 2.15.0 using the ‘metafor’ (Viechtbauer, 2010) and ‘nlme’ (Pinheiro et al., 2013) packages.

Table 2.1. Definitions applied for the main life history traits of *O. ostertagi* and equations used for their calculation.

	Larval establishment	**Hypobiosis**	**Adult mortality**	**Female fecundity**
Definition	Proportion of ingested larvae that develops to adult stage.	Proportion of ingested larvae that goes in hypobiosis.	Proportion of adult worms that die per day.	Number of eggs produced by an adult female per day.
Estimate	$\frac{AWB}{ID}$	$\frac{eL_4}{ID}$	$\frac{-\ln\left(\frac{AWB}{ID}\right)}{t}$ *	$\frac{FEC_n \times DFP}{AWB \times F}$
Variance	$\frac{var(AWB)}{ID^2}$	$\frac{var(eL_4)}{ID^2}$	$\frac{Var(AWB)}{(t \times AWB)^2}$	$\frac{DMP^2}{F^2} \times \frac{FEC^2}{AWB^2} \times \left(\frac{var(FEC)}{FEC^2} + \frac{var(AWB)}{AWB^2}\right)$
Specific eligibility criteria	Duration of infection between 21 and 40 days.	Time lag between (last) infection and necropsy at least 2 weeks.	Duration of infection greater than 40 days.	Mono-infections with *O. ostertagi.*

List of abbreviations used: AWB = Adult worm burden; FWB = Female worm burden; eL_4 = Larvae in the early L_4 stage; ID = Infection dose; FEC_n = Faecal egg count at necropsy; DFP= Daily faeces production; F = Proportion of females; t = days after (first) infection.
* based on Smith et al., 1994.

2A.3 Results

2A.3.1 Systematic review and study inclusion

The search in ISI Web of Science using general keywords yielded 5266 publications. A title based selection of all these publications resulted in 404 publications that were considered potentially relevant. A second full text based selection gave 111 publications that met the inclusion criteria. Publications were read in English, German, French and Spanish. Finally, ninety-five papers provided sufficient data to perform quantitative analysis for at least one of the four life history parameters of *O. ostertagi*. Figure 2.2 provides an overview of the systematic review results, including the reasons for exclusion.

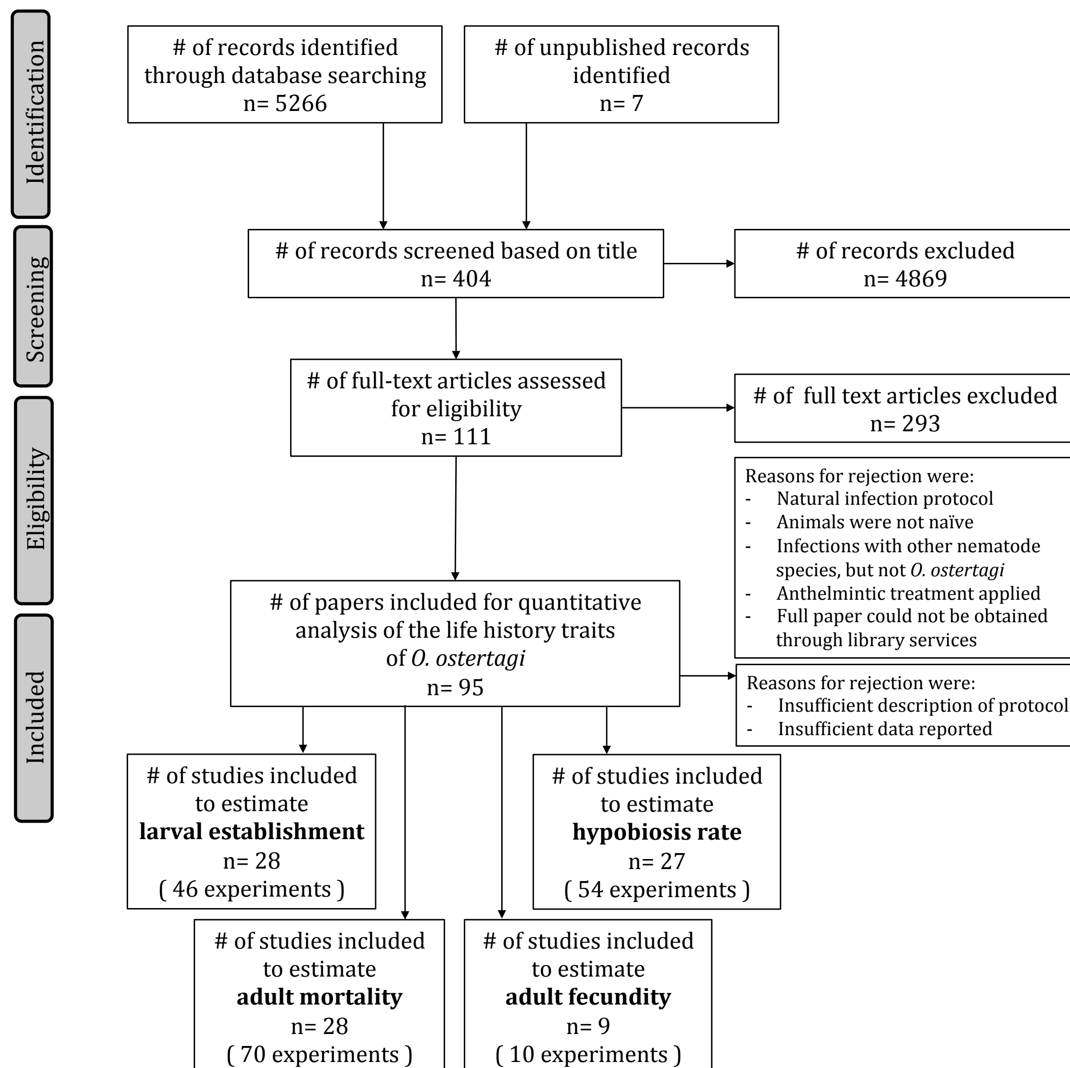

Figure 2.2. Flowchart of systematic review of the main life history traits of the parasitic phase of *O. ostertagi* and study selection for meta-analysis. Adapted from PRISMA (Moher et al., 2009).

2A.3.2 Study characteristics

The main breed of animals in the selected data was Holstein or a crossing with this breed, the age of animals ranged from 2 to 24 months. The database to calculate the larval establishment rate of *O. ostertagi* contained 26 peer-reviewed publications, amounting to a total of 44 experiments. Additionally, two unpublished UGent trials were added to this database (Table 2.2). The database to estimate the proportion of ingested *O. ostertagi* larvae going into hypobiosis contained 21 peer-reviewed publications and 6 unpublished UGent trials, resulting in a total of 54 experiments (Table 2.3). The database to calculate adult mortality consisted of 28 studies and 70 experiments. Five of these were unpublished UGent trials, while the others were derived from peer-reviewed publications (Table 2.4). The obtained database for the calculation of the female fecundity consisted of 9 studies totalling 10 experiments. Six studies were peer-reviewed studies, while the other three were unpublished UGent trials (Table 2.5). As stated by the specific eligibility criteria for this latter trait, only experiments in which animals received a mono-infection with *O. ostertagi* were taken into account. However, an exception was made for a study in which animals received a concomitant infection with *Dictyocaulus viviparus*, since hosts excrete larvae rather than eggs of this species and therefore *O. ostertagi* eggs could be differentiated from *D. viviparus* larvae in faeces.

The separate database to estimate the average proportion of female worms present in the abomasum was generated, containing individual data from 75 experimental animals originating from 6 different studies.

2A.3.3 Meta-analysis: Larval establishment

An average (± S.E.) larval establishment rate of 0.269 (± 0.022) was found (Figure 2.3). The establishment of *O. ostertagi* was associated with both the infection dose (P<0.001) and host age at the time of first infection (P=0.025). A smaller proportion of larvae reached the adult phase when a large number of L_3 was ingested (Figure 2.4a) and when animals were younger at the time of first infection (Figure 2.4b). The full mixed effects model explained 43% of the total amount of heterogeneity. The results of the final

mixed effects model to estimate the establishment of *O. ostertagi* are provided in Table 2.6.

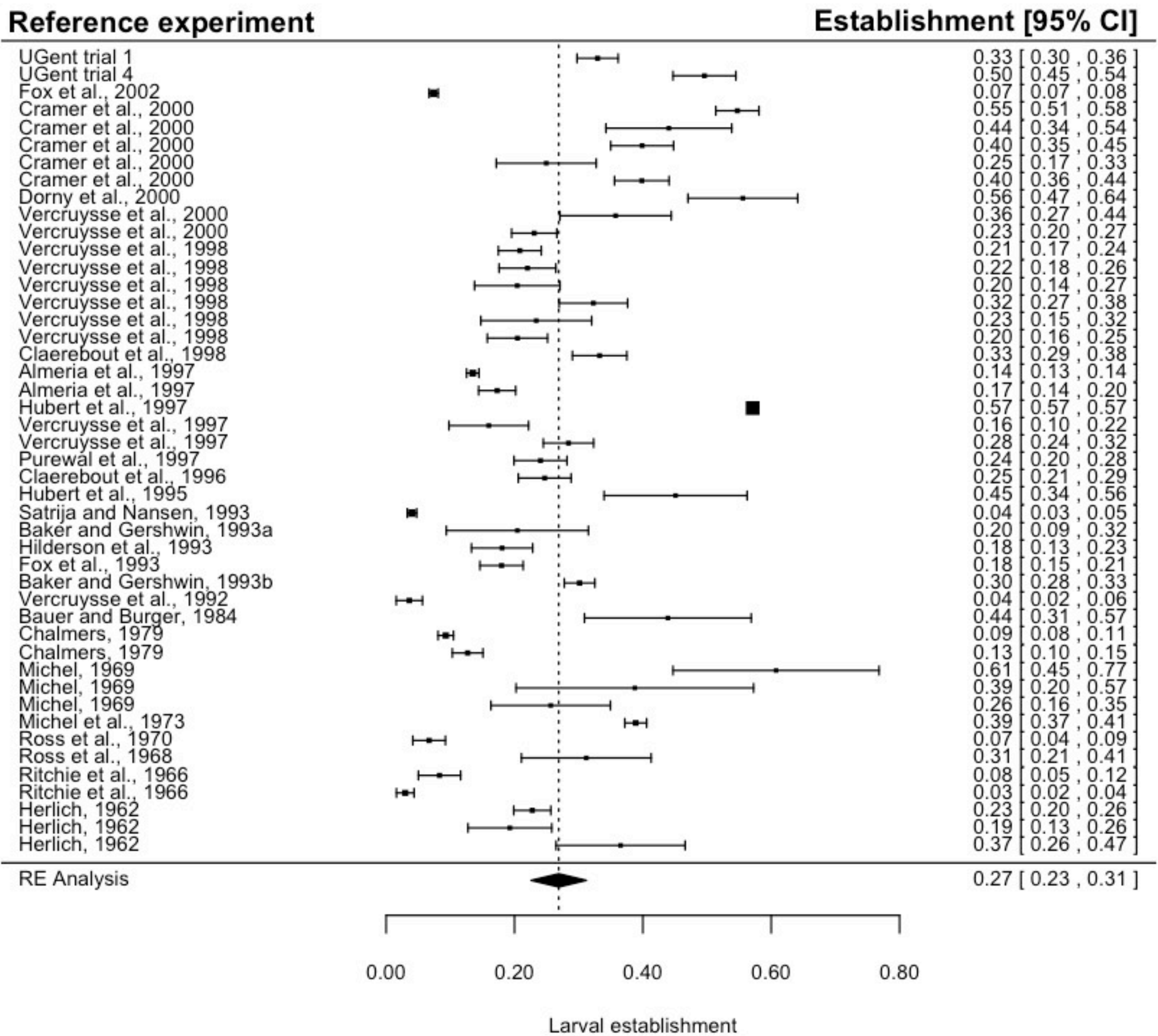

Figure 2.3 Meta-analysis estimating the larval establishment rate of *O. ostertagi* using a random-effects (RE) analysis. Rectangles represent the establishment for each experiment. Size of the rectangles represents the weight given to each experiment in the analysis based on the precision of each study effect measure. Error bars correspond to the 95% CI.

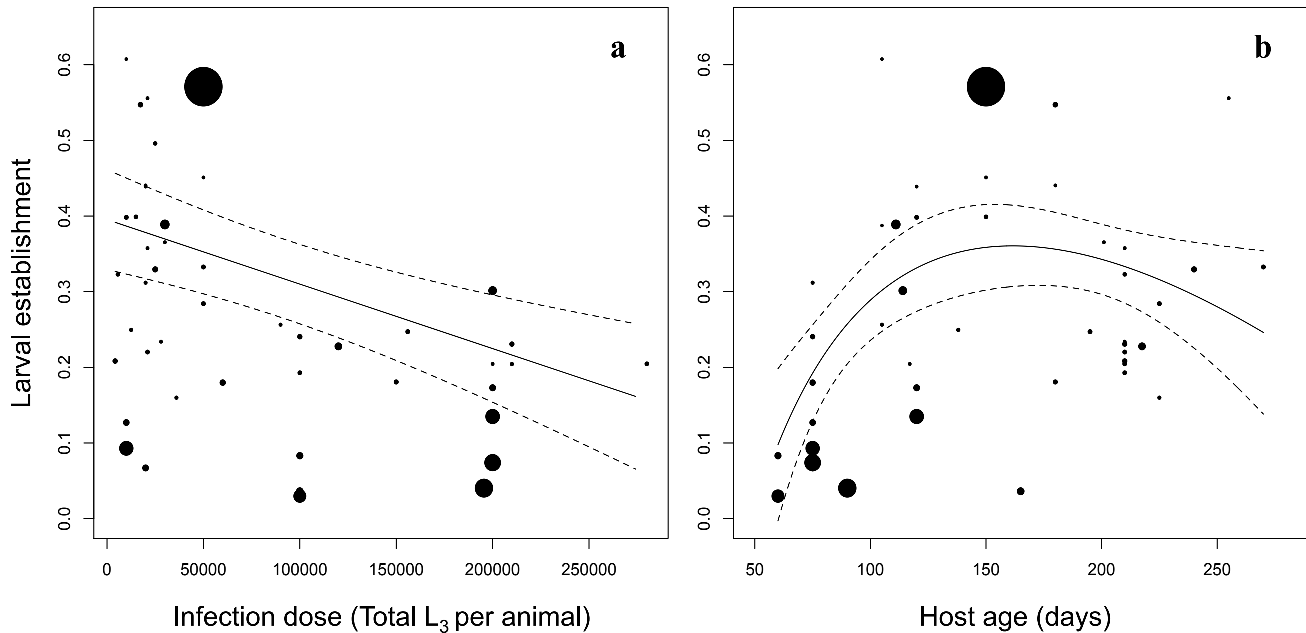

Figure 2.4 Predicted average larval establishment of *O. ostertagi* (a) as a function of infection dose at median host age (150 days) and (b) as a function of host age (days) at median infection dose (43000 L_3 per animal). Predictions are based on the equation of the final mixed model for establishment that can be found in Table 2.6. Black dots represent average establishment for each study. Size of the dots marks the weight given to each study based on the precision of each study effect measure. Dashed bars correspond to the 95% CI of the mean.

2A.3.4 Meta-analysis: Hypobiosis

The hypobiosis rate in naïve calves ranged between zero and 0.307. An average (± S.E.) hypobiosis rate of 0.041 (± 0.009) was computed (Figure 2.5). The proportion of ingested larvae entering arrested development was significantly affected by whether or not animals received concomitant infections with other nematode species besides *O. ostertagi* (mixed vs. mono infection; P<0.001). The final mixed effects model explained 30% of the total amount of heterogeneity. The results of the final mixed effects model to estimate the hypobiosis rate of *O. ostertagi* are provided in Table 2.6.

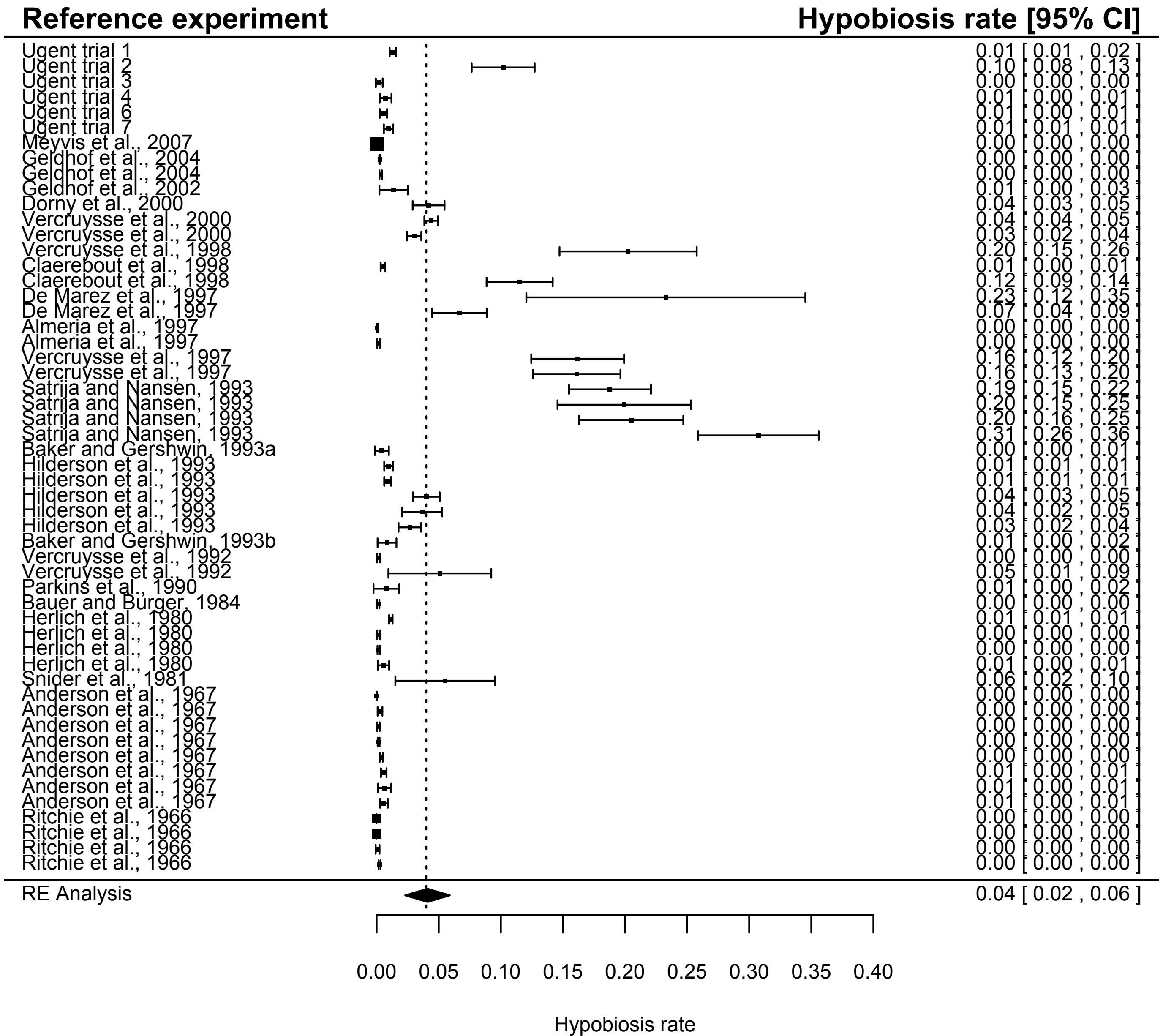

Figure 2.5 Meta-analysis estimating the hypobiosis rate of *O. ostertagi* using a random-effects (RE) analysis. Rectangles represent the establishment for each experiments. Size of the rectangles represents the weight given to each experiment in the analysis based on the precision of each study effect measure. Error bars correspond to the 95% CI.

2A.3.5 Meta-analysis: Adult mortality

An average (± S.E.) daily instantaneous per capita death rate of 0.028 ± 0.002 was computed. Adult worm mortality was significantly influenced by infection dose ($P<0.001$) and duration of infection ($P=0.019$). However, these two moderators were significantly correlated in our dataset ($P<0.001$, Spearman's R = 0.45). To assess density dependence of adult mortality, infection dose was kept in the final model (Figure 2.6). The resulting mixed effect model explained 33% of the total amount of heterogeneity. The results of the final mixed effects model to estimate the adult mortality of *O. ostertagi* for trickle infections are provided in Table 2.6.

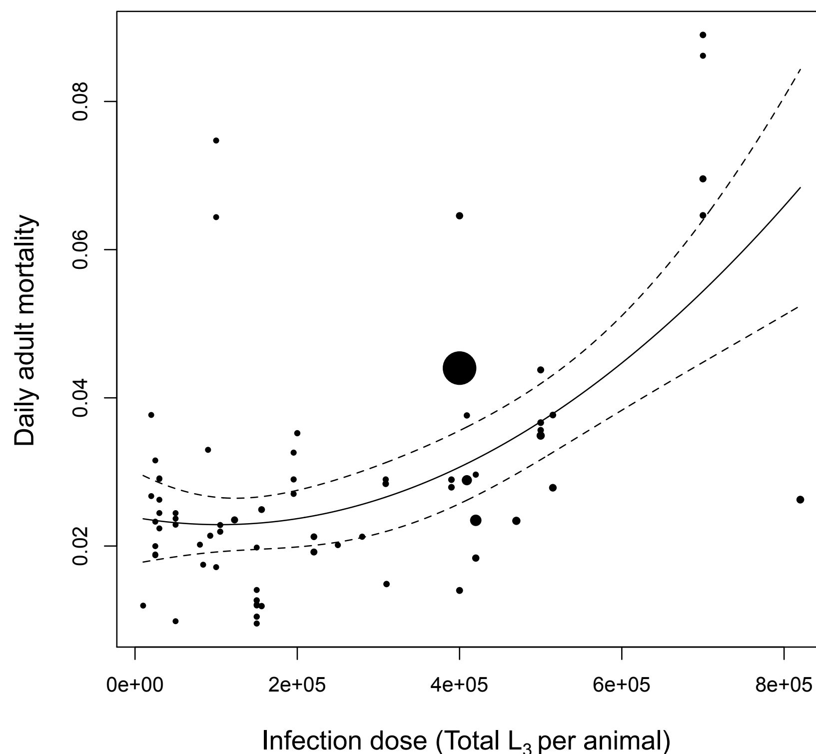

Figure 2.6 Predicted average adult mortality of *O. ostertagi* as a function of the infection dose. Predictions are based on the equation of the final mixed model for adult mortality that can be found in Table 2.6. Size of the dots marks the weight given in the analysis to each study based on the precision of each study effect measure. Dashed bars correspond to the 95% CI of predictions.

Table 2.6 The results of the final mixed analysis models to estimate several life history traits of the parasitic phase of *O. ostertagi*.

	Variable	Estimate	S.E.	*P*
Larval establishment rate				
	Intercept	-2.57	0.86	0.002
	Age in days	-0.004	0.002	0.006
	Log(Age in days)	0.73	0.22	0.001
	Infection dose	-0.008×10^{-4}	0.002×10^{-4}	<0.001
Hypobiosis				
	Intercept	0.09	0.01	<0.001
	Mono infection (vs. mixed infection)	-0.07	0.02	<0.001
Adult mortality rate				
	Intercept	0.024	0.004	<0.001
	Infection dose	-0.002×10^{-5}	0.002×10^{-5}	0.445
	Infection dose^2	0.009×10^{-11}	0.003×10^{-11}	0.011

2A.3.6 Meta-analysis: Female fecundity

The average (± S.E.) proportion of *O. ostertagi* females found in the abomasum was 0.545 (± 0.017). Both infection dose (P=0.048) and duration of infection (P=0.004) were positively associated with the proportion of female worms present. Because these two moderators were correlated (P<0.0001; Pearson R = 0.76), only duration of infection was retained as an independent variable explaining *O. ostertagi* sex ratio in further calculations of fecundity.

The average daily fecundity (± S.E.) was 284 (± 45) eggs per female (Figure 2.7), what corresponds to an average of 0.025 (± 0.004) eggs per gram faeces per female when daily faeces production is not taken into account. Because this dataset was limited and homogenous, no moderators could be evaluated.

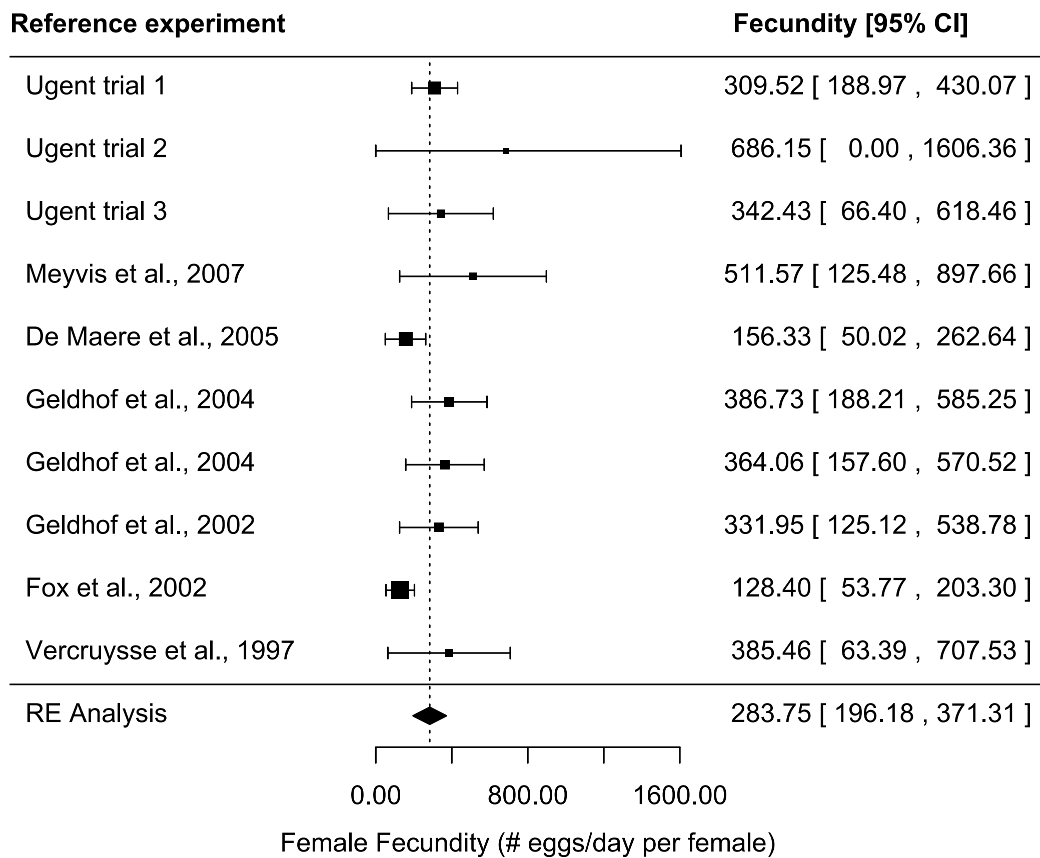

Figure 2.7 Meta-analysis estimating the daily female fecundity of *O. ostertagi* using a random-effects (RE) analysis. Rectangles represent the daily fecundity for each experiment. Size of the rectangles represents the weight given to each experiment in the analysis based on the precision of each study effect measure. Error bars correspond to the 95% CI.

Table 2.2 Characteristics of studies that were used to assess the larval establishment rate of *O. ostertagi*.

Reference	Number of experiments	Number of animals	Breed	Average age (days)	Nematode species	Country	Protocol	Infection dose *O. ostertagi*	Number of infections	Duration of infection
Almeria et al., 1997	2	6	Holstein	120	Oo	USA	Single	200000	1	21-28
Baker and Gershwin, 1993a	1	4	Holstein	117	Oo	USA	Single	200000	1	35
Baker and Gershwin, 1993b	1	2	Holstein	114	Oo	USA	Single	200000	1	35
Bauer and Bürger, 1984	1	4	German Black Pied	120	Oo/Co	Germany	Single	20000	1	22
Chalmers, 1979	2	7	Friesian	75	Oo/Co	New Zealand	Trickle	10000	4	24-28
Claerebout et al., 1996	1	6	Holstein	195	Oo	Belgium	Trickle	156000	12	35
Claerebout et al., 1998	1	6	Holstein-Friesian	270	Oo/Co	Belgium	Trickle	50000	10	31
Cramer et al., 2000	5	27	Holstein/ Anguscross/ Fleckvieh/ Friesian/ Hereford cross	154	Oo/Oer/Dv/ Hp/Hc/Co/ Cp/Tc/Ta/ Nh/Cspp.	USA/ Germany/UK /Australia	Single	10000-20000	1	29
Dorny et al., 2000	1	7	Holstein cross	255	Oo/Co	Belgium	Trickle	21000	21	34
Fox et al., 1993	1	3	Jersey	75	Oo	UK	Single	60000	1	28
Fox et al., 2002	1	4	Friesian	75	Oo	UK	Single	200000	1	28
Herlich, 1962	3	10	Holstein/Jersey	210	Oo/Cp/ Ta/Tc	USA	Single	30000-120000	1	35-37
Hilderson et al., 1993	1	4	NA	180	Oo	Belgium	Trickle	150000	10	31
Hubert et al., 1995	1	5	Montbéliard	150	Oo/Dv	France	Single	50000	1	22
Hubert et al., 1997	1	5	Montbéliard	150	Oo/Dv	France	Single	50000	1	21

Michel et al., 1973	1	3	Friesian	111	Oo	UK	Trickle	30000	30	33
Michel, 1969	3	12	NA	105	Oo	UK	Single	10000-90000	1	25
Purewal et al., 1997	1	4	Friesian	75	Oo	UK	Single	100000	1	28
Ritchie et al., 1966	2	4	Ayrshire	60	Oo	UK	Single	100000	1	21-28
Ross, 1968	1	3	Friesian/ Friesian cross	75	Oo/Co/Dv	USA	Single	20000	1	23
Ross, 1970	1	4	Friesian	75	Oo/Cp	USA	Single	20000	1	24
Satrija and Nansen, 1993	1	3	Jersey	90	Oo/Co	Denmark	Single	195500	1	28
UGent trial 1	1	4	Holstein cross	240	Oo	Belgium	Trickle	25000	25	35
UGent trial 4	1	8	Holstein	300	Oo/Co/Dv	Belgium	Single	25000	1	32
Vercruysse et al., 1992	1	4	Holstein cross	165	Oo/Co	Belgium	Single	100000	1	28
Vercruysse et al., 1997	2	14	Holstein cross	225	Oo/Dv	Belgium	Trickle	36000-50000	18-25	22-35
Vercruysse et al., 1998	6	35	Holstein cross	210	Oo/Co	Belgium	Trickle	5600-280000	21-28	26-33
Vercruysse et al., 2000	2	12	Holstein cross	210	Oo/Co	Belgium	Trickle	21000-210000	21	25-32

List of abbreviations used: NA= Not available, Oo = *Ostertagia ostertagi*, Co = *Cooperia oncophora*, Cp = *Cooperia punctata*, Cspp. = *Cooperia* spp., Dv = *Dictyocaulus viviparus*, Oer = *Oesophagostomum radiatum*, Hc = *Haemonchus contortus*, Hp = *Haemonchus placei*, Tc = *Trichostrongylus colubriformis*, Ta = *Trichostrongylus axei*, Nh = *Nematodirus helvetianus*.

Table 2.3 Characteristics of studies that were used to assess the hypobiosis rate of *O. ostertagi*.

Reference	Number of experiments	Number of animals	Breed	Average age (days)	Nematode species	Country	Protocol	Infection dose *O. ostertagi*	Number of infections	Duration of infection
Almeria et al., 1997	2	6	Holstein	120	Oo	USA	Single	200000	1	21-28
Anderson et al., 1967	8	24	NA	64	Oo	UK	Single/ Trickle	20000-820000	1-20	42-136
Baker and Gershwin, 1993a	1	4	Holstein	117	Oo	USA	Single	200000	1	35
Baker and Gershwin, 1993b	1	2	Holstein	114	Oo	USA	Single	200000	1	35
Bauer and Bürger, 1984	1	4	German Black Pied	120	Oo/Co	Germany	Single	20000	1	22
Claerebout et al., 1998	2	16	Holstein-Friesian	270	Oo/Co	Belgium	Trickle	50000-550000	10-120	31-206
De Marez et al., 1997	2	6	Holstein	210	Oo	Belgium	Trickle	156000-576000	12-36	44-194
Dorny et al., 2000	1	7	Holstein cross	255	Oo/Co	Belgium	Trickle	21000	21	34
Geldhof et al., 2002	1	6	Montbéliard	210	Oo	Belgium	Trickle	25000	25	59
Geldhof et al., 2004	2	13	Montbéliard	240	Oo	Belgium	Trickle	25000	25	59
Herlich et al., 1980	4	12	Holstein-Friesian	319	Oo	USA	Single	600000	1	60
Hilderson et al., 1993	5	20	NA	180	Oo	Belgium	Trickle	150000-870000	10	31-175
Meyvis et al., 2007	1	7	Holstein cross	240	Oo	Belgium	Trickle	25000	25	56
Parkins et al., 1990	1	6	Friesian	120	Oo/Co	UK	Trickle	84000	42	84
Ritchie et al., 1966	4	8	Ayrshire	60	Oo	UK	Single	100000	1	21-90
Satrija and Nansen, 1993	4	12	Jersey	90	Oo/Co	Denmark	Single/ Trickle	150000-195500	1-41	28-168

Snider et al., 1981	1	2	Holstein	NA	Oo	USA	Trickle	470000	24	122
UGent trial 1	1	4	Holstein cross	240	Oo	Belgium	Trickle	25000	25	35
UGent trial 2	1	7	NA	255	Oo	Belgium	Trickle	30000	30	59
UGent trial 3	1	7	Crossbred	330	Oo	Belgium	Trickle	30000	30	51
UGent trial 4	1	8	Holstein	300	Oo/Co/Dv	Belgium	Single	25000	1	32
UGent trial 6	1	7	Holstein	NA	Oo	Belgium	Trickle	30000	30	64
UGent trial 7	1	10	Holstein	NA	Oo/Co	Belgium	Single	25000	1	42
Vercruysse et al., 1992	2	8	Holstein cross	165	Oo/Co	Belgium	Single/ Trickle	100000-280000	1-36	28-168
Vercruysse et al., 1997	2	14	Holstein cross	225	Oo/Dv	Belgium	Trickle	36000-50000	18-25	22-35
Vercruysse et al., 1998	1	6	Holstein cross	210	Oo/Co	Belgium	Trickle	280000	28	33
Vercruysse et al., 2000	2	12	Holstein cross	210	Oo/Co	Belgium	Trickle	21000-210000	21	25-32

List of abbreviations used: NA= Not available, Oo = *Ostertagia ostertagi*, Co = *Cooperia oncophora*, Dv = *Dictyocaulus viviparus*.

Table 2.4 Characteristics of studies that were used to assess the daily adult mortality of *O. ostertagi*.

Reference	Number of experiments	Number of animals	Breed	Average age (days)	Nematode species	Country	Protocol	Infection dose *O. ostertagi*	Number of infections	Duration of infection
Anderson et al., 1967	8	24	NA	64	Oo	UK	Single/ Trickle	20000-820000	1-20	42-136
Burden et al., 1978	4	20	Friesian	75	Oo	UK	Trickle	105000-220500	70-147	70-147
Claerebout et al., 1998	1	10	Holstein-Friesian	270	Oo/Co	Belgium	Trickle	50000	10	206
De Maere et al., 2005	1	7	Montbéliard	300	Oo	Belgium	Trickle	30000	30	57
De Marez et al., 1997	2	6	Holstein	210	Oo	Belgium	Trickle	156000-576000	12-63	44-194
Geldhof et al., 2002	1	6	Montbéliard	210	Oo	Belgium	Trickle	25000	25	59
Geldhof et al., 2004	2	13	Montbéliard	240	Oo	Belgium	Trickle	25000	25	59
Herlich et al., 1980	4	12	Holstein-Friesian	319	Oo	USA	Single	600000	1	60
Hilderson et al., 1993	4	16	NA	180	Oo	Belgium	Trickle	150000	10	168-175
Hilderson et al., 1995	3	12	Crossbred	180	Oo/Co	Belgium	Trickle	150000	51	136
Li et al., 2010	1	4	Holstein	105	Oo	USA	Single	100000	1	190
MacPherson et al., 1987	4	12	Friesian	90	Oo	UK	Trickle	700000	12	90-120
Meyvis et al., 2007	1	7	Holstein cross	240	Oo	Belgium	Trickle	25000	25	56
Michel et al., 1973	3	11	Friesian	111	Oo	UK	Trickle	80000-250000	30-250	81-251
Michel et al., 1979	10	36	Friesian	533	Oo	UK	Trickle	92700-515000	103	108
Michel, 1969	3	11	NA	105	Oo	UK	Single	10000-90000	1	50
Parkins et al., 1990	1	6	Friesian	120	Oo/Co	UK	Trickle	84000	42	84
Ritchie et al., 1966	2	4	Ayrshire	60	Oo	UK	Single	100000	1	60-90

Ross and Dow, 1964	1	2	NA	180	Oo	UK	Trickle	200000	2	105
Satrija and Nansen, 1993	6	18	Jersey	90	Oo/Co	Denmark	Trickle	50000-195500	40-41	140-168
Snider et al., 1981	1	2	Holstein	NA	Oo	USA	Trickle	470000	24	122
UGent trial 2	1	7	NA	255	Oo	Belgium	Trickle	30000	30	59
UGent trial 3	1	7	Crossbred	330	Oo	Belgium	Trickle	30000	30	51
UGent trial 5	1	10	NA	NA	Oo/Tc/Nh	Belgium	Single	20000	1	41
UGent trial 6	1	7	Holstein	NA	Oo	Belgium	Trickle	30000	30	64
UGent trial 7	1	10	Holstein	NA	Oo/Co	Belgium	Single	25000	1	42
Vercruysse et al., 1992	1	4	Holstein cross	165	Oo/Co	Belgium	Trickle	280000	36	168
Wiggin and Gibbs, 1987	1	4	Holstein	120	Oo	USA	Trickle	310000	7	112

List of abbreviations used: NA= Not available, Oo = *Ostertagia ostertagi*, Co = *Cooperia oncophora*, Tc = *Trichostrongylus colubriformis*, Nh = *Nematodirus helvetianus*.

Table 2.5 Characteristics of studies that were used to assess the female fecundity of *O. ostertagi*.

Reference	Number of experiments	Number of animals	Breed	Average age (days)	Nematode species	Country	Protocol	Infection dose *O. ostertagi*	Number of infections	Duration of infection
De Maere et al., 2005	1	7	Montbéliard	300	Oo	Belgium	Trickle	30000	30	57
Fox et al., 2002	1	4	Friesian	75	Oo	UK	Single	200000	1	28
Geldhof et al., 2002	1	6	Montbéliard	210	Oo	Belgium	Trickle	25000	25	59
Geldhof et al., 2004	2	13	Montbéliard	240	Oo	Belgium	Trickle	25000	25	59
Meyvis et al., 2007	1	7	Holstein cross	240	Oo	Belgium	Trickle	25000	25	56
UGent trial 1	1	4	Holstein cross	240	Oo	Belgium	Trickle	25000	25	35
UGent trial 2	1	7	NA	255	Oo	Belgium	Trickle	30000	30	59
UGent trial 3	1	7	Crossbred	330	Oo	Belgium	Trickle	30000	30	51
Vercruysse et al., 1997	1	7	Holstein cross	225	Oo/Dv	Belgium	Trickle	50000	25	35

List of abbreviations used: NA= Not available, Oo = *Ostertagia ostertagi*, Dv = *Dictyocaulus viviparus*.

2A.4 Discussion

The need for quantification of life history traits to parameterize mechanistic models is a bottleneck in their development. Also, quantification of uncertainty in parameter estimates is relevant to interpretation of model outputs and identification of knowledge gaps. The estimates of main life history traits together with their uncertainty provided in this study will help to improve the precision of future models describing *O. ostertagi* epidemiology. Incorporating variation in host and parasite factors influencing these estimates will help to make future model predictions more robust under changing conditions and scenarios.

A great benefit of systematic review combined with meta-analysis is the use of existing data in making new parameter estimates, even though the original studies pursued other research goals (Lean et al., 2009). In the protocol applied a number of choices were made. First, no specific search for “grey literature”, such as conference proceedings, was included. However, unpublished data from the Laboratory of Parasitology (Faculty Veterinary Medicine, Ghent University, Belgium) provided important additional information. Secondly, analysis of aggregated study data was allowed, because otherwise an important number of studies would not have met eligibility criteria, resulting in a small, limited dataset. An exception was made to calculate the sex ratio of *O. ostertagi,* which was based solely on the analysis of individual data. Third, the assumption was made that the efficacy of the techniques used for worm recovery was similar across the different studies. However, due to the lack of standardization, under- or overestimation of the actual worm burden might be present (Eysker and Kooyman, 1993). Finally, many studies on infections with *O. ostertagi* in cattle reported only geometric mean worm and/or egg counts. Without raw data, conversion to the arithmetic mean was impossible and these studies were excluded from further analysis.

Due to technical constraints on identifying ingested cohorts of larvae (Georgi and Le Jambre, 1983), tracking how numbers of different parasite life stages exactly change during an infection, has posed a challenge to parasitologists for many years. The typical course of a trichostrongylid

infection, such as *O. ostertagi*, in ruminants, shows a decline in worm burden as the infection proceeds (Smith, 1994). This decrease is assumed to be a result of two effects: increasing parasite mortality and a reduced proportion of parasites reaching the adult stage. Both effects are known to be a consequence of host immunity (Barger, 1987), but caution is needed in disentangling each to quantify them. Analysis of data from Michel (1970) shows that the number of *O. ostertagi* surviving in time follows a declining sigmoid function (Smith, 1994). Therefore, it was assumed in the current study that for a short period, namely 40 days following the start of infection, there is no or very little adult worm mortality. Consequently, larval establishment and adult mortality were estimated from studies with a duration of infection of respectively less and more than 40 days. This was a compromise between gathering sufficient data and limiting the inevitable confounding between adult parasite mortality and reduced establishment of larval stages, as infection proceeds.

Evidence exists that both confirms (Dunsmore, 1960; Ross, 1963; Anderson and Michel, 1977) and denies (Michel, 1970; Barger, 1987) density dependence of establishment and mortality of *O. ostertagi*. The present study provides evidence that both traits are indeed density dependent since the magnitude of the rate was associated with the total number of infective larvae given to the animals. When one wants to extrapolate the given results to natural infections, it is important to limit the scope to realistic ranges of daily ingested L_3 numbers. Based on analysis of calf tracer data of Shaw et al. (1998), animals on infected pastures in western Europe ingest on average 263 and 4365 L_3 per day at the start and end of the pasture season respectively (Verschave, unpublished analysis). In our database, the daily infection dose of *O. ostertagi* was on average 22013 L_3 and ranged between 200 and 100000 L_3. The higher end of this range might therefore represent conditions that are rarely encountered in the field, but possibly still relevant in the context of future climatic and management change.

Most studies investigating the influence of host age on the establishment of *O. ostertagi* report no effect of age on the number of worms established (Michel et al., 1973; Herlich, 1980; Kloosterman et al., 1991). Michel et al. (1979) however found that acquired resistance to establishment

of *O. ostertagi* develops more slowly in animals of 133 days than in older animals. A new finding in the current study was that the proportion of larvae establishing successfully was lowest in the youngest calves, increased up to an age of 100 days and then levelled off (Figure 2.4b). This may be explained as an effect of maternal immunity that slowly fades after birth, but could also be associated with the physiological development of the rumen. Different mechanisms can prevent ingested trichostrongylid larvae from reaching the adult stage. On one hand, development of exsheathed L_3 can be prevented due to exclusion from entering the abomasal mucosa in immune animals (Smith et al., 1994). On the other hand, failure of L_3 to exsheath in the rumen before they enter the abomasum is described as a cause of rapid initial loss after ingestion in helminth-naïve animals (Dakkak et al., 1981; Smith et al., 1994). The ruminal pH is highest in neonatal calves and gradually declines in the first 8 weeks as the rumen matures (Beharka et al., 1998). Because the ruminal pH plays an important role in the exsheathment of L_3 (Sommerville, 1957; Rogers and Sommerville, 1960; Davey and Rogers, 1982; DeRosa et al., 2005), this might explain the lower establishment in young animals. Finally, in young calves, the reticular groove reflex is activated by ingestion of fluids causing bypass of the rumen (Reece, 2009). Deposition of sheathed L_3 straight into the abomasum due to this process might also result in lower establishment rates.

In the current study, hypobiosis rates were significantly higher in animals that received concomitant infections with other nematode species besides *O. ostertagi* (mixed infections). Theoretically, a synergistic effect of cross immunity to different nematode species could explain this, as host immunity is one of the important inducers of arrested development. However, studies investigating the existence of cross immunity between GIN contradict this hypothesis (Satrija and Nansen, 1993; Hilderson et al., 1995; Dorny et al., 1997). Additional influence on the number of larvae entering hypobiosis of other factors described in literature could not be assessed based on the data in this study, but are expected to play a role in both experimental and natural infections. In experimental infections, storage conditions of L_3 prior to infection are expected to have an important influence for example. For natural infections, the declining environmental temperature at the end of the grazing

season in temperate regions, for example, is widely assumed to be associated with the commonly increased proportion of *O. ostertagi* larvae found in hypobiosis during winter (Eysker, 1997).

For *O. ostertagi*, Hansen and Perry (1994) report a daily egg production of 100 - 200 eggs per female. Analysis of Smith et al. (1987b) describes an initial fecundity ranging between 234 and 1232 eggs laid per day per female depending on infection dose and infection type (single vs. trickle). Even though faecal egg counts are often assessed during infection trials and necropsies, many studies did not report them directly (geometric means or depicted in graphs) resulting in a dataset with little variation between experiments. An accurate estimate of fecundity and how it is modified during the course of an infection by immunity or parasite density (Smith et al., 1987b) is crucial in parameterizing mechanistic models for *O. ostertagi* because the predicted pasture infectivity depends on it. Data on the number of eggs *in utero* of *O. ostertagi* females can also be found in literature. However, use of these data as a reliable indicator of daily fecundity and the effect of immunity on this trait is questionable. Immune responses reduce worm length and shorter worms have fewer eggs (Claerebout and Vercruysse 2000), but there is no known correlation between the number of eggs *in utero* and the number of eggs excreted daily. A last impediment in assessing female fecundity was the limited data available that describes daily fresh faeces production of cattle in function of bodyweight. Further research is needed in this area to obtain more precise information on realistic predictions of daily faecal excretion across a full range of ages and diets in cattle.

Compared to male worms, the number of female worms present increased significantly with increasing infection dose. Because infection dose was positively correlated with the duration of infection, due to trickle infections, this either indicates that females live longer than males or are less affected by density dependence created by resource competition or immune response. These findings of a female-biased sex ratio coincide with similar reports in other nematode species (e.g. Waller and Thomas, 1987; Craig et al., 2010), but have, to our knowledge, not been previously documented for *O. ostertagi*.

In conclusion, this systematic review is the first to collect and summarize available data to estimate the four main life history traits of the parasitic phase of *O. ostertagi.* The meta-analysis provides novel average estimates and information on their variation to parameterize life cycle based transmission models. More accurate models will improve our understanding of parasite epidemiology, help to focus research and assist targeted worm control in cattle adjusted to future needs. This study also supports existing evidence for density dependence of larval establishment and adult mortality, shows that host age affects the establishment rate and provides the first evidence of a female-biased sex ratio for *O. ostertagi*. The complete effect of acquired immunity on the dynamics of the parasitic phase of *O. ostertagi*, however, is still not fully understood. The lack of information to quantify the influence of immunity on fecundity and the insufficient data on older and ‘fully’ immune animals in the current study need to be addressed by future research.

2A.5 References

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PART B. QUANTIFICATION OF THE PARASITIC PHASE FOR *C. ONCOPHORA*.

Adapted from

Verschave S.H., Rose H., Morgan E., Claerebout E., Vercruysse J., Charlier J. Modelling Cooperia oncophora: quantification of key parameters in the parasitic phase, submitted to Veterinary Parasitology.

2B.1 Introduction

Mathematical models that simulate transmission dynamics of GIN infections have already been around for several decades in the field of veterinary parasitology (e.g. Gordon et al., 1970; Gettinby et al. 1979; Grenfell et al., 1987; Barnes and Dobson, 1990). Given the variety of factors influencing GIN infections (e.g. climate, parasite-host interactions) such models can be essential tools to represent and manipulate such systems in ways that would not be possible or practical in the field (Scott and Smith, 1994). In the coming decades, parasitic disease patterns are expected to change due to the impact of climate change and the growing issue of anthelmintic resistance (van Dijk et al., 2010). The nature and impact of these changes, however, is difficult to foresee. Parasite transmission models enable the extrapolation of current knowledge to alternative scenarios and can therefore enhance our understanding of parasite epidemiology under changing conditions (Rose et al., 2015). Moreover, they play an important role in obtaining insights in the development of anthelmintic resistance (Gettinby et al., 1989; Barnes and Dobson, 1990) and underpin the search for alternative control strategies (Smith et al., 1987; Charlier et al., 2014). Because of its high prevalence and pathogenicity, *O. ostertagi* has been the primary focus of transmission models developed to simulate GIN infections in cattle (Gettinby et al., 1979; Grenfell et al., 1987; Chaparro et al., 2013; Rose et al., 2015). Key life history traits of the parasitic phase of *O. ostertagi* were recently quantified through meta-analysis to facilitate the development and parameterisation of future transmission models (Verschave et al., 2014). However, in the light of the development of anthelmintic resistance, *C. oncophora*, another highly abundant nematode of cattle, gains impact as a dose-limiting species for the most commonly used anthelmintics (Sutherland and Leathwick, 2011). Despite its growing importance, no transmission model for this nematode species has yet been developed. Moreover, little information is available on the population dynamics of *C. oncophora*, which is crucial for the development and parameterisation of specific nematode transmission models. Here, we provide the results of a systematic review and meta-analysis that quantifies the main life history traits of the parasitic phase

of *C. oncophora* and investigates potential influences of immunity on these traits.

2B.2 Materials and methods

The four life history traits of the parasitic phase of *C. oncophora* addressed were: (1) the pre-adult mortality, (2) the adult mortality, (3) the hypobiosis factor and (4) the female fecundity. The pre-adult and adult mortality are respectively defined as the instantaneous daily per capita death rate of pre-adult and adult stages, loosely interpreted as the proportion that die per day. The hypobiosis factor is defined as the proportion of ingested larvae that enters arrested development, and the female fecundity represents the number of eggs produced by a female worm each day. The search strategy and eligibility criteria used to perform the systematic review were the same as those described by Verschave et al. (2014) and section 2A.2. In short, studies in which naïve bovines were artificially infected with *C. oncophora* using a single or trickle infection protocol and that reported worm counts after necropsy with an associated measure of variance were identified in peer-reviewed publications and unpublished studies carried out at the Laboratory of Parasitology (Ghent University, Belgium (UGent)). The Web of Knowledge database was last searched using specific keywords ((cattle OR bovine OR cow OR heifer OR bull OR steer OR calf OR calves) AND (nematode OR helminth OR parasit∗ OR trichostrongyl∗ OR ostertag∗ OR Cooperia OR oncophora) AND (infect∗ OR transm∗)) on February 6, 2012. Exclusion criteria are shown in Figure 2.8. Methods used to estimate the pre-adult mortality of *C. oncophora* were the same as those used to calculate the establishment rate for *O. ostertagi* in Verschave et al. (2014) and section 2A.2. Methods used to estimate the adult mortality, hypobiosis factor and female fecundity of *C. oncophora* were the same as those used in Verschave et al. (2014) and section 2A.2. Due to the absence of sufficient data, the average proportion of *C. oncophora* females present in the small intestine was calculated based on the average numbers of females found for each experiment, rather than the numbers of females found in the individual animals as described by Verschave et al. (2014) and section 2A.2. All other

data extraction, effect measure calculations and meta-analyses were performed as described by Verschave et al. (2014) and section 2A.2. The equations used for the calculation of each life history trait and corresponding variances are given in Table 2.7. To investigate heterogeneity between experiments the following moderator variables were evaluated: infection protocol (single or trickle infection), infection dose (total number of L3 given per animal), duration of infection (days), host age (days) and whether or not animals received concomitant infections with nematode species other than *C. oncophora* (mixed or mono infection).

Table 2.7. Equations used for the calculation of selected life history traits of the parasitic phase of *C. oncophora* using meta-analysis, and the obtained estimates.

Parameter	Specific eligibility criteria concerning the duration of infection (days)	Estimate	Variance	Inverse variance weighted average	95% CI
Pre-adult mortality	21-40	$\frac{-ln(\frac{AWB}{ID})}{t}$	$\frac{var(AWB)}{t \times AWB}$	0.044	0.037 – 0.052
Adult mortality	>40	$\frac{-ln(\frac{AWB}{ID})}{t}$	$\frac{var(AWB)}{t \times AWB}$	0.039	0.031 – 0.048
Hypobiosis	>21	$\frac{eL4}{ID}$	$\frac{Var(eL4)}{ID^2}$	0.007	0.004 – 0.011
Female fecundity	>21	$\frac{(FEC \times DFP)}{(AWB \times F)}$	$\frac{DFP^2}{F^2} \times \frac{FEC^2}{AWB^2} \times (\frac{var(FEC)^2}{FEC^2} + \frac{var(AWB)^2}{AWB^2})$	2744	1146 – 4342
Proportion of females (F)	>21	$\frac{FEM}{AWB}$	$\frac{FEM^2}{AWB^2} \times (\frac{var(FEM)}{FEM^2} + \frac{var(AWB)}{AWB^2})$	0.534	0.494 – 0.573

Note: AWB= Adult worm burden; ID = Infection dose; t = duration of infection; eL4 = early L4 stages; FEC = faecal egg count at necropsy; DFP = Daily faeces production; F = Proportion of females; FEM = Number of adult female worms. Pre-adult mortality was estimated on duration of infection of 21-40 days to allow for establishment without being unduly affected by adult mortality (See Verschave et al. (2014) and section 2A.2 for a full explanation).

2B.3 Results

After title-based and full text-based selection, 49 publications met the inclusion criteria (Figure 2.8). Of these, 22 publications, including 3 unpublished UGent studies, provided sufficient data to perform the quantitative analysis for at least one of the four life history traits (Figure 2.8; Figure 2.9). Data originated from 2.5 – 9 month old Holstein Friesian or Holstein Friesian cross cattle. A summary of the study characteristics can be found in Table 2.8. The average proportion of female worms present in the small intestine was based on data from 17 experiments reported in 3 peer-reviewed publications and 2 unpublished UGent trials.

The average (95% confidence interval (CI)) pre-adult mortality was 0.044 (0.037–0.052) (Table 2.7). This pre-adult mortality was positively associated with the infection dose administered to the animals (P = 0.022). The full mixed effects model (0.036 (±0.005) + infection dose x 0.104 x 10^{-6} (±0.045 x 10^{-6})) explained 20% of the total amount of heterogeneity in the dataset used to estimate pre-adult mortality. The average (95% CI) adult mortality was 0.039 (0.031–0.048) (Table 2.7) and was not significantly associated with any of the tested moderator variables. An average (95% CI) hypobiosis factor of 0.007 (0.004-0.011) was computed (Table 2.7). The proportion of ingested larvae entering hypobiosis was not significantly affected by any of the tested moderator variables. The average (95% CI) proportion of *C. oncophora* females found in the small intestine of the animals was 0.534 (0.494-0.573). The average female fecundity (95% CI) was 2744 (1146-4342) eggs per female per day (Table 2.7). The female fecundity was negatively correlated with infection dose (P = 0.033). The full mixed effects model (4000 (±952) - infection dose x 0.016 (±0.008)) explained 25% of the total amount of heterogeneity found in the dataset. A detailed forest plot for each of the life history trait can be found in Figures 2.9 and 2.10.

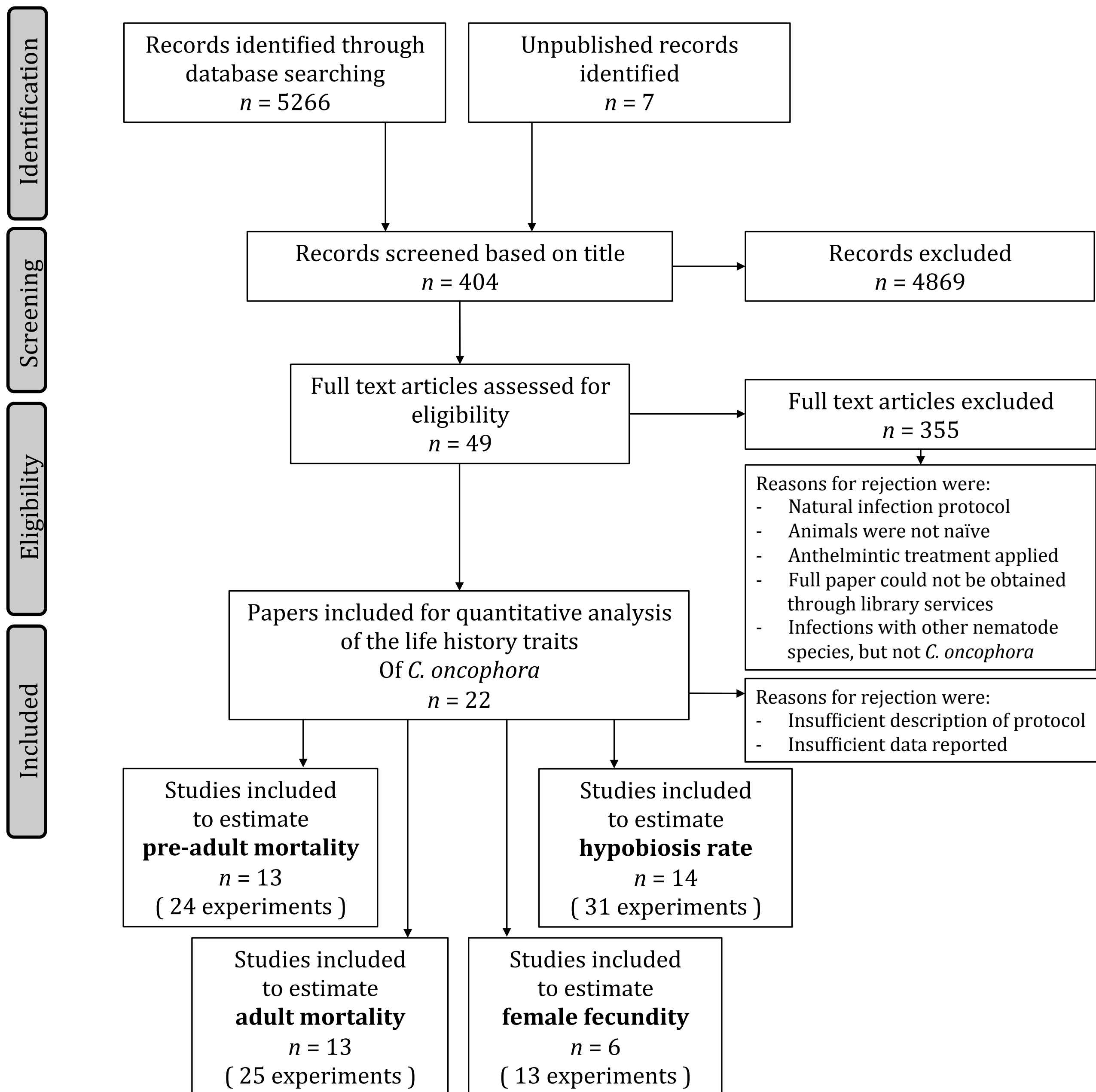

Figure 2.8. Flowchart of the systematic review of key life history traits of the parasitic phase of *C. oncophora* and exclusion criteria for study selection to perform the meta-analysis. Adapted from PRISMA (Moher et al., 2009).

Table 2.8. Characteristics of the studies used to estimate key life history traits of the parasitic phase of *C. oncophora.*

	Pre-adult mortality	**Adult mortality**	**Hypobiosis**	**Female fecundity**
Number of animals	117	117	143	77
Average age in days (range)	150 (75 – 270)	135 (90 – 270)	143 (90 – 270)	205 (75 – 270)
Gender				
Males	16	20	28	9
Females	4	3	0	3
Unknown	4	2	3	1
Average infection dose (range)	83808 (4200 – 210000)	272840 (20000 – 2000000)	239548 (20000 – 2000000)	72369 (4200 – 280000)
Average duration of infection in days (range)	29 (24 – 33)	100 (41 – 206)	78 (24 – 206)	42 (22 - 206)
Infection type				
Single	11	11	16	2
Trickle	13	14	15	11
Mono	8	17	21	0
Mixed	16	8	10	13

Note: The terms mixed and mono refer to whether or not animals received concomitant infections with nematode species other than *C. oncophora.*

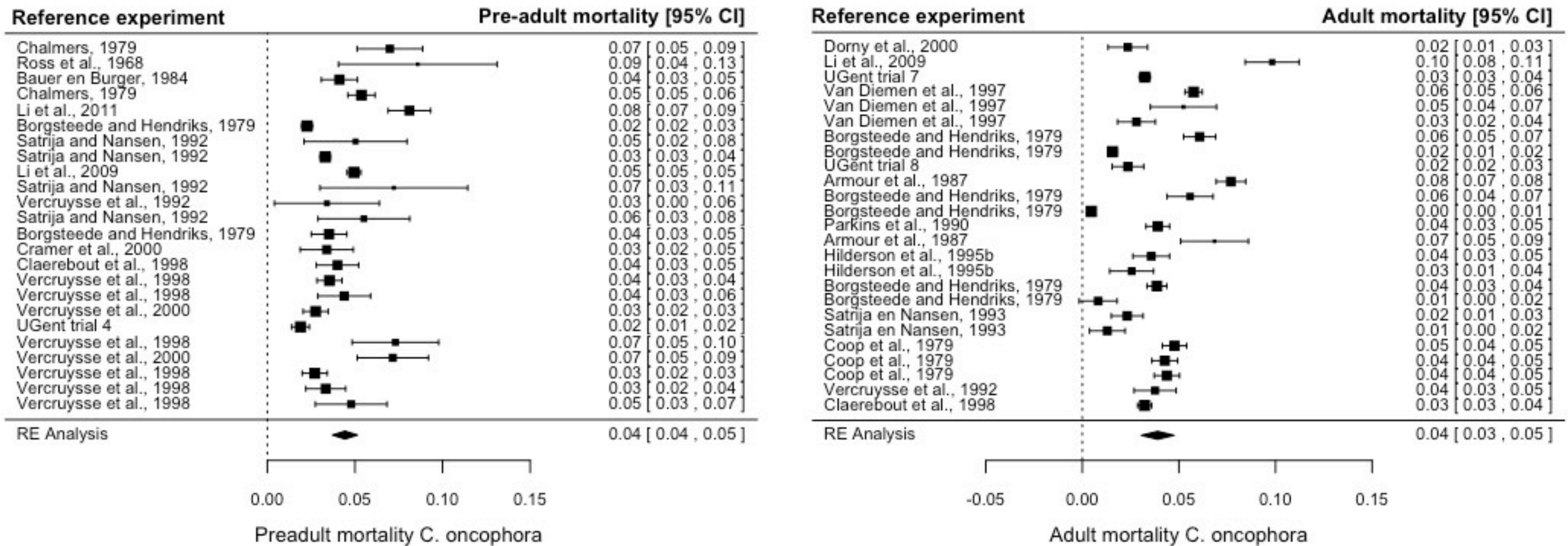

Figure 2.9 Meta-analysis estimating key life history traits of the parasitic phase for *C. oncophora* (pre-adult and adult mortality) using a random-effects (RE) analysis. Rectangles represent the effect measure for each experiment. Size of the rectangles represents the weight given to each experiment in the analysis based on the precision of each study effect measure. Error bars correspond to the 95% CI.

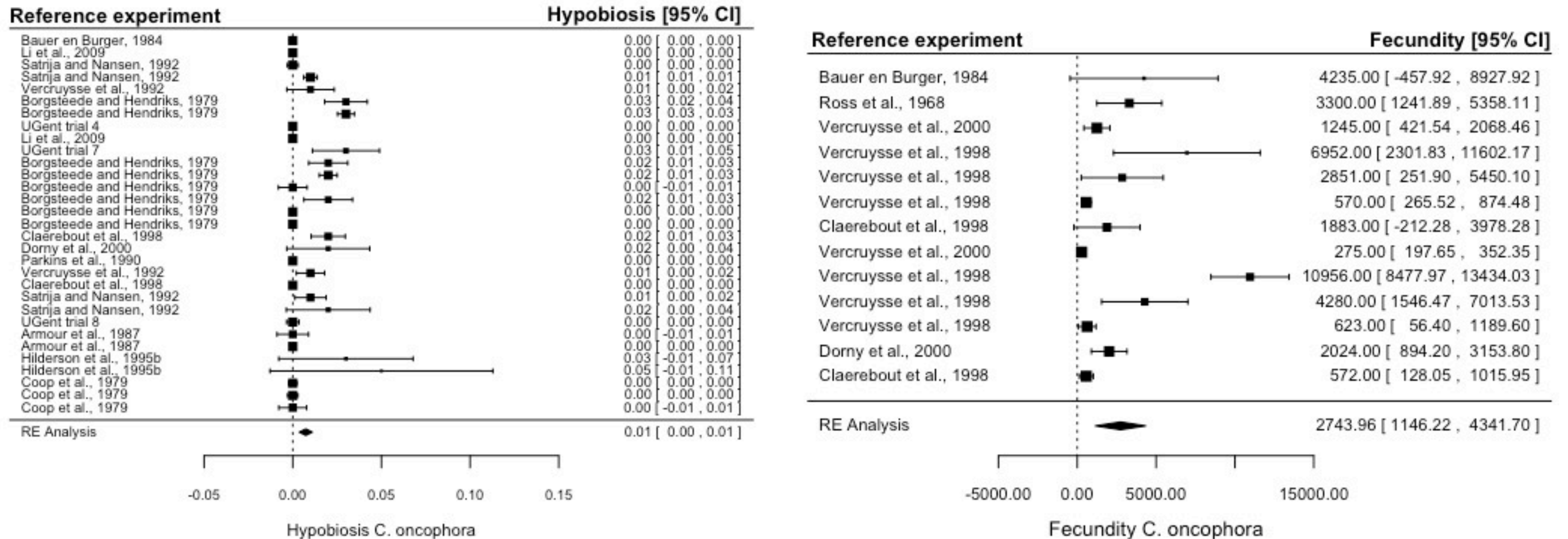

Figure 2.10 Meta-analysis estimating key life history traits of the parasitic phase for *C. oncophora* (hypobiosis and fecundity) using a random-effects (RE) analysis. Rectangles represent the effect measure for each experiment. Size of the rectangles represents the weight given to each experiment in the analysis based on the precision of each study effect measure. Error bars correspond to the 95% CI.

2B.4 Discussion

Systematic review and meta-analysis enable bundling research efforts of previous decades to provide an extensive base for parameter estimation. Publication bias is potentially associated with these techniques and incorporation of unpublished experiments in the current study aimed to mitigate this. Identification of knowledge gaps in the literature is a second asset of systematic review. The collected data was restricted to dairy cattle breeds, which might limit the external validity to beef cattle. Also, most experiments infected male animals instead of female calves and no eligible data were available for animals older than 9 months.

No explicit estimates of the pre-adult and adult mortality of *C. oncophora* are available in the literature for comparison with the currently estimated values. Different studies do report a sudden drop in worm numbers during the course of both natural and artificial infections with *C. oncophora* around 9 to 12 weeks after first exposure, indicating a sharp increase in worm mortality around that time (Kloosterman et al., 1991; Smith and Archibald, 1968). Others state that worm expulsion in most animals occurs earlier, at 6 weeks after first infection, but that a large variation in the ability to develop an effective immune response exists between individuals (Kanobana et al., 2001; 2002). Pre-adult mortality was positively correlated with infection dose in our study, suggesting that the establishment of ingested *C. oncophora* larvae is affected by either density-dependent processes or the level of acquired immunity. This is in contrast with the findings of Kanobana et al. (2004), who found no difference in establishment rate between cattle exposed to different infection levels of *C. oncophora*. The fact that no significant correlation was found between pre-adult mortality and host age confirms previous findings that the resistance to larval establishment appears to be acquired rather than age-associated (Smith and Archibald, 1968). Also for adult mortality no correlation with host age was found in the current study. The exact effect of host age on resistance to *C. oncophora* has yet to be elucidated and contradictory evidence exists in the literature. Both Armour (1989) and Kloosterman et al. (1991) state that the ability to acquire effective immunity against *C. oncophora* increases with host age. Kloosterman et al. (1991)

found a significantly lower worm burden in calves inoculated at 6 months of age compared to calves inoculated at 3 months of age. The worm burden of calves inoculated at 9 months of age, however, was higher compared to that of calves inoculated at 6 months of age, which weakens their statement of age-dependent immunity development. Smith and Archibald (1968) found smaller worms containing fewer eggs in older animals compared to younger animals that had the same level of contact, but both groups showed comparable levels of worm numbers at necropsy. The average hypobiosis factor estimated for *C. oncophora* was lower than that estimated for *O. ostertagi* (Verschave et al., 2014). The analysis did not provide evidence of significant moderators related to immunity affecting the entry of *C. oncophora* into hypobiosis. A fecundity of 1000 to 3000 eggs produced per day per female has been reported for *C. oncophora* (Hansen and Perry, 1994), which is comparable with the average estimate of female fecundity found in the current meta-analysis. It is possible that both studies under-estimate actual egg production, as egg recovery efficiency during the enumeration of faecal egg density is unknown. A large variation in fecundity existed between the individual experiments, ranging from 275 to 10,956 eggs produced per day per female. A negative correlation was found between fecundity and infection dose, suggesting that either density dependent processes or the level of acquired immunity affect the number of eggs produced per female. Similarly, other researchers found that female worms derived from the distal segment of the small intestine carried significantly lower numbers of eggs when animals had experienced a higher level of infection with *C. oncophora* (Kanobana et al., 2004).

A lack of data on parasite population dynamics is a common problem encountered in the development and parameterisation of transmission models that describe parasite life cycles. Also for *C. oncophora*, a highly abundant cattle nematode of increasing importance, detailed knowledge of the population dynamics is scarce. The current systematic review and meta-analysis provides robust estimates for key traits of the parasitic phase of *C. oncophora*, which should now be fed into transmission models for this parasite in order to facilitate the evaluation of alternative control approaches.

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CHAPTER 3

MODELLING THE PARASITIC PHASE

Adapted from

Rose H.[*], Verschave S.H.[*], Morgan E., Claerebout E., Vercruysse J., Fisher, M., Fenn, C., Charlier J. GLOWORM-PARA: A flexible model framework for the parasitic phase of gastro-intestinal nematode parasites in ruminants. Submitted to International Journal for Parasitology.

**equal contribution*

3.1 Introduction

The production of sufficient quantities of safe and good quality food is a global concern (Fitzpatrick, 2013). In light of this, animal health management needs to be optimised in livestock farm systems to underpin efficient and economic production. GINs are an important threat to food security and economic livestock farming as they cause significant production losses in ruminants (Nieuwhof and Bishop, 2005; Charlier et al., 2009). Currently, the control of GIN infections in livestock is primarily based on the chemotherapeutic use of anthelmintic substances (Charlier et al., 2014). However, both the development of anthelmintic resistance and the influence of climate change on farm management and parasite epidemiology are expected to challenge the future control of these infections (Morgan and van Dijk, 2012; Skuce et al., 2013). Progress has been made towards targeted, sustainable control strategies that are economically sound (Charlier et al., 2014) but the need for adequate decision-support tools to aid in the implementation of these strategies remains (Morgan et al., 2013). The epidemiology of GIN infections is a result of complex interactions between parasite, host, climate, farm management and historic control strategies. Parasite transmission models are therefore indispensable as they provide the potential to include a variety of processes on different levels and extrapolate current knowledge to alternative scenarios at large temporal scales (Rose et al., 2015).

The development of mathematical models to simulate the transmission dynamics of GIN infections in ruminants dates back several decades. The majority of the existing models have been developed specifically for GIN infections in sheep (e.g. Callinan et al., 1982; Dobson et al., 1990; Leathwick et al., 1992; 1995; Bishop and Stear, 1997) and a much smaller number of models exists for cattle (Gettinby et al., 1979; Gettinby and Paton, 1981, Grenfell et al., 1987a; Ward, 2006; Chaparro et al., 2013). The cattle models have tended to only focus on one nematode species, i.e. *O. ostertagi*, perhaps due to its pathogenic significance compared with *C. oncophora*, against which cattle develop and mount an effective immune response. No single-species model exists for *C. oncophora* despite its increasing

importance in the context of anthelmintic resistance (Sutherland and Leathwick, 2011). Generic models that provide a framework for GIN infections that can be applied to a range of hosts and GIN species are also scarce, while their development is of great interest in identifying emergent patterns of change (Molnar et al., 2013). Recently, a generic model framework for the free-living phase of GIN in ruminants that has important modifications on behaviour and development of the GIN on pasture, was developed (GLOWORM-FL, Rose et al., 2015). To explore the consequences of different control and management approaches on parasite epidemiology, however, a complementary model for the parasitic phase is needed as host-parasite interactions and host acquired immunity play a crucial part in the transmission dynamics of GINs (Claerebout and Vercruysse, 2000).

Despite decades of model development, certain challenges remain in replicating the parasitic phase of the GIN lifecycle. Host behaviour, for example, may be an important driver of the infection risk to GINs (Fox et al., 2013). Yet, a limited number of models incorporate behavioural aspects and no model has considered the grazing behaviour of ruminants in relation to the vertical distribution of infective larvae on herbage swards. The mathematical incorporation of acquired immunity and its impact on parasite population dynamics has also challenged modellers over the past years (Cornell, 2005). A generally accepted approach is to assume that acquired immunity increases with exposure to infective L3 and wanes during periods in which the infection rate is low (Roberts and Grenfell, 1991). Others have simulated the immune response to exposure (Singleton et al., 2011). The inability to directly quantify the level of acquired immunity against GINs and the absence of realistic parameter estimates, however, remains a problem and prevents a biologically meaningful mechanistic description and parameterisation of the immune response.

The aim of the current study was to develop a conceptual model framework for the parasitic phase, GLOWORM-PARA, that can be applied to a range of GIN species. The model is parameterised and validated for two species that are of major importance in cattle, i.e. *O. ostertagi* and *C. oncophora.* Different approaches to parameterise the development of acquired immunity against GINs were compared and the framework

incorporates host grazing behaviour. An extensive set of field observations of first season grazing cattle was used for model validation.

3.2 Materials and methods

3.2.1 GLOWORM-PARA model framework

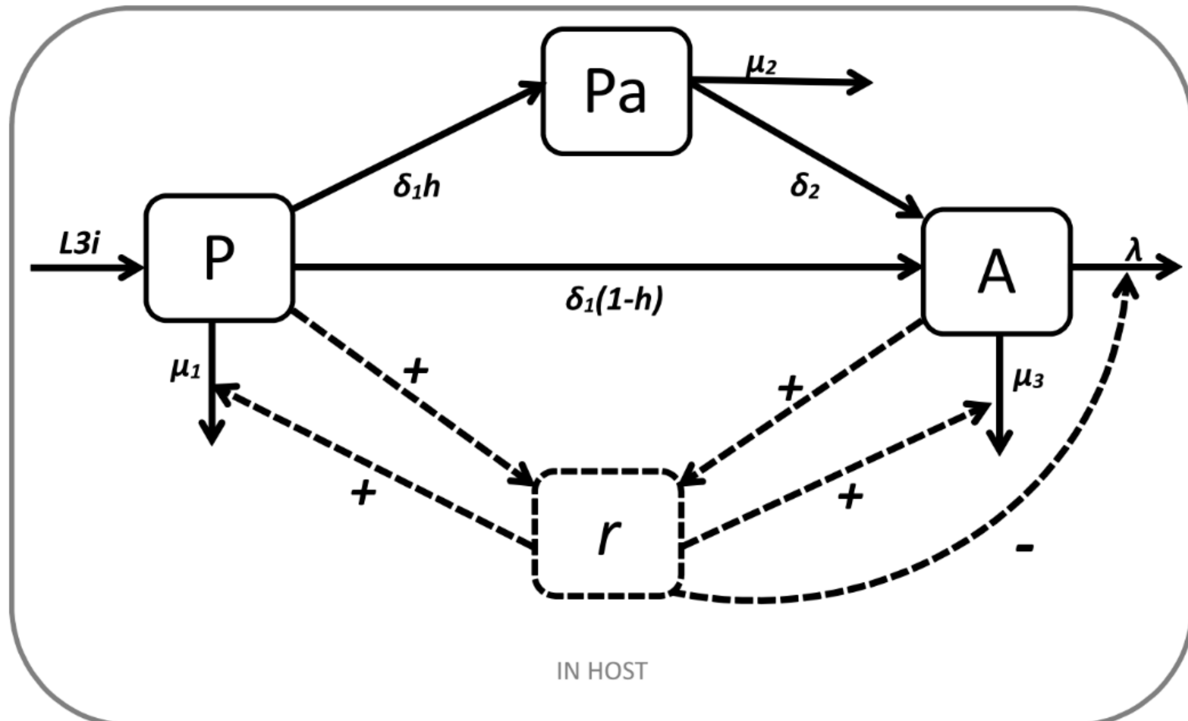


Figure 3.1 Conceptual framework of the GLOWORM-PARA model. State variable and parameter definitions are given in Table 3.1. Positive and negative symbols indicate the direction of moderation of parameters e.g. increasing acquired immunity (r) has a negative impact on fecundity (λ) but increases mortality rates (μi).

The model framework is based on the life cycle of the parasitic stages of trichostrongylid nematodes (Figure 3.1) and tracks the mean number of nematodes and level of acquired immunity in a group of hosts. Infective third stage larvae, L_3, are ingested with herbage (*L3i*) and enter the pool of pre-adult parasitic nematodes (*P; equation 1*). Pre-adult nematodes develop to adult nematodes (*A*) either directly (equation 3) or via arrested (hypobiotic) L_4 (*Pa; equation 2*). Acquired immunity (*r*) increases in response to exposure to pre-adult (*P*), including ingested L_3 (*L3i*), and adult nematodes (*A*) and decays with time (equation 4). Arrested L4 do not contribute to the development or

maintenance of immunity (Miller and Horohov, 2006). State variables and model parameters are defined in Table 3.1.

$$\frac{dP}{dt} = L3i - \delta_1 P - \mu_1(L3i + P) \quad (1)$$

$$\frac{dPa}{dt} = -(\delta_2 + \mu_2)Pa + \delta_1 hP \quad (2)$$

$$\frac{dA}{dt} = \delta_1(1 - h)P + \delta_2 Pa - \mu_3 A \quad (3)$$

$$\frac{dr}{dt} = \rho(P + A)(1 - r) - \sigma r \quad (4)$$

Mean faecal egg counts (*FEC*; eggs per gram) for the group of hosts can be estimated from the number of adults (*A*), proportion of adults that are female (pA_f), daily fecundity (λ) and expected daily faeces production (*f; equation 5*).

$$FEC = \frac{(pA_f)A\lambda}{f} \quad (5)$$

Table 3.1 State variable and parameter definitions.

State variable / Parameter	Definition	Units
P	Pre-adult nematodes in the host (L3, L4 and immature adults)	-
Pa	Arrested L4	-
A	Mature adults	-
r	Acquired immunity (resistance)	-
L3i	Number of L3 ingested	day^{-1} $host^{-1}$
δ_1	Development rate from ingested L3 to mature adult	P^{-1} day^{-1}
δ_2	Development rate from arrested L4 to mature adult	Pa^{-1} day^{-1}
μ_1	Pre-adult mortality rate	P^{-1} day^{-1}
μ_2	Arrested L4 mortality rate	Pa^{-1} day^{-1}
μ_3	Adult mortality rate	A^{-1} day^{-1}
h	Proportion of developing pre-adult nematodes entering hypobiosis (arrested development)	Proportion
ρ	Immune response	$(P+A)^{-1}$ day^{-1}
σ	Immune decay in the absence of exposure to infection	Day^{-1}
pA_f	Proportion of adults that are female	Proportion
λ	Daily fecundity (eggs produced)	Eggs $female^{-1}$ day^{-1}
f	Daily faeces production	Grams (wet weight) day^{-1}

3.2.2 Model integration

The model was implemented in R (R Core Team, 2014) using the "lsoda" function of the "deSolve" package (Soetaert et al., 2010) for solving differential equations. The model returns daily output. Treatments were implemented using the "events" argument of the "lsoda" function, assuming 100% treatment efficacy. Model output is the mean worm burden and egg output per host, which can be used to estimate faecal egg count (eggs per gram) if faeces production is known. Variation between individuals is not simulated and therefore the model is a "mean-field" model.

3.2.3 Parameter estimates

The model framework was parameterised for two economically important species infecting cattle: the abomasal nematode *O. ostertagi* and the intestinal nematode *C. oncophora*. However, the generic framework can be applied to any trichostrongylid nematode in ruminants using the same methods described here.

Constant rates

The development rate from ingested L3 to mature adult was estimated from species-specific prepatent periods of 17.5 days (*O. ostertagi*) and 18.1 days (*C. oncophora;* Table 3.2). These prepatent periods were estimated from the average time to maturity of *O. ostertagi* and *C. oncophora* in helminth-naïve calves, which was assumed to be the midpoint between the first appearance of eggs in faeces and peak egg output (Appendix A).

No data were available in the literature to estimate the mortality rates of arrested L4 due to the confounding effects of resumed development. Therefore, the mortality rate of arrested L4 for both *O. ostertagi* and *C. oncophora* was set at 0.002 after Grenfell et al. (1987a). Mortality rates of all other pre-adult and adult nematodes were a function of immunity (see section '*Immunity-mediated regulation of the parasite population*').

The proportion of adults that are female (pA_f) was previously estimated for both species by systematic review and meta-analysis (Verschave et al. 2014; Verschave et al., submitted; Table 3.2).

Table 3.2 Parameter estimates for *Ostertagia ostertagi* (Oo) and *Cooperia oncophora* (Co).

Parameter	*Species*	*Estimate*	*Source*
δ_1	Oo	-ln(0.5)/17.5 = 0.040	Current study (Appendix A)
	Co	-ln(0.5)/18.1 = 0.038	Current study (Appendix A)
$\mu_{1(min)}$	Oo	0.054	Verschave et al. (2014)
	Co	0.044	Verschave et al., submitted
$\mu_{1(max)}$	Oo	0.062	Verschave et al. (2014)
	Co	0.052	Verschave et al., submitted
μ_2	Oo, Co	0.002	Grenfell et al. (1987a)
$\mu_{3(min)}$	Oo	0.028	Verschave et al. (2014)
	Co	0.039	Verschave et al., submitted
$\mu_{3(max)}$	Oo	0.032	Verschave et al. (2014)
	Co	0.048	Verschave et al., submitted
$h_{(min)}$	Oo	0.02	Verschave et al. (2014)
	Co	0.004	Verschave et al., submitted
$h_{(max)}$	Oo	0.06	Verschave et al. (2014)
	Co	0.011	Verschave et al., submitted
ρ	Oo	2.28×10^{-7}	Current study (estimated)
		0.0695	Current study (fitted)
	Co	3.32×10^{-7}	Current study (estimated)
		0.1493	Current study (fitted)
σ	Oo, Co	0.002	Current study (expert opinion)
pA_f	Oo	0.545	Verschave et al. (2014)
	Co	0.534	Verschave et al., submitted
$\lambda_{(min)}$	Oo	196	Verschave et al. (2014)
	Co	1253	Verschave et al., submitted
$\lambda_{(max)}$	Oo	284	Verschave et al. (2014)
	Co	2968	Verschave et al., submitted

Development of acquired immunity

Acquired immunity against GINs increases during the course of an infection, however, direct quantification of acquired immunity and rate of increase is difficult (Claerebout and Vercruysse, 2000). Parameterising the response rate (ρ), i.e. the increase in acquired immunity with exposure to GINs,in the model was therefore a challenge and two different approaches were compared, i.e. parameter estimation versus parameter fitting (Table 3.2).

<u>Estimated response rate</u>

The rationale for the estimation of the response rate was to assess the level of exposure needed to achieve protective immunity for *O. ostertagi* and *C. oncophora*, respectively. The average number of L3 ingested per day over the course of a grazing season was calculated using raw data from field trials across Europe collected by Shaw et al. (1998). The data concerned worm counts from naïve tracer calves that had grazed 'clinical' pastures (i.e. pastures on which an outbreak of parasitic gastroenteritis in the untreated first season grazers was seen) during two weeks at the start and the end of the grazing season respectively (Shaw et al., 1998). A larval establishment of 27% and 29% was taken into account for *O. ostertagi* and *C. oncophora,* respectively, to calculate the average number of L3 ingested per day per animal from the total worm burden at necropsy (Verschave et al., 2014; Verschave et al., submitted). Secondly, the total amount of larvae ingested over an average grazing season was calculated using the following simplifying assumptions: (1) an average grazing season for young stock lasts 6 months (Charlier et al., 2010) and (2) the number of L3 ingested per day for the first and second half of the grazing season, corresponds to the values calculated for the start and end of the season, respectively. Finally, it was assumed that protective immunity (r=1) was typically acquired after 9 months (1.5 grazing seasons) and 6 months (1 grazing season) of exposure for *O. ostertagi* and *C. oncophora*, respectively (Armour, 1989; Ploeger et al., 1995; Claerebout et al., 1998; Ravinet et al., 2014), and the response rate was estimated as: *1/(total L3 ingested x number grazing seasons)*.

Fitted response rate

As an alternative approach, the response rate was fitted to an independent set of data originating from published and unpublished field trials. These trials were all performed in Belgium between 1994 and 2013 and concerned first season grazing cattle that were naturally infected with GINs on pasture. Cattle varied between 4.5 and 10 months of age at turnout and group size varied between 10 and 14 animals (Table 3.3). Daily pasture contamination values for the entire period of each trial were obtained by polynomial interpolation of pasture contamination values observed during the trials. From this, the daily intake rate of L3 per host was calculated using dry matter intake estimates based on the average age of the animals (see section '*Host grazing behaviour, dry matter intake and faecal production*'). The daily L3 intake data were then used as model input and GLOWORM-PARA model simulations were run for each trial to predict the mean daily *O. ostertagi* and *C. oncophora* egg output per host. The daily faecal production per host was estimated using the average animal weight (see section '*Host grazing behaviour, dry matter intake and faecal production'*) at turn out reported in the trials and used to calculate average daily faecal egg count (eggs per gram of faeces) from the simulation output. The response rate was then fitted using an optimisation procedure which minimises the sum of squared errors between faecal egg counts observed in the trials and predicted faecal egg counts (using the "optim" function in R).

Immune decay rate

Acquired immunity is assumed to decay with time (Roberts and Grenfell, 1991). No data were available for formal estimation of this decay rate. However, expert judgement (n=3) placed the estimated decay rate over an average 6 month housing period (Charlier et al 2010) at between 10% and 50%. Therefore, a 6-month decay rate of 30% was used to estimate a daily decay rate (σ; Table 3.2).

Table 3.3 Details concerning the data of the field trials used to fit the immune response parameter. These field trials were also combined with the field observations from 2012-2013 (Table 3.4) to validate the model parameterised using the immune response parameter estimated from Shaw et al. (1998). Approximate stocking rates are shown where the values are known.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Source	Vlaminck et al., 2015 (experiment 2)	Unpublished	Unpublished	Unpublished	Unpublished	Unpublished
Location	Melle	Waregem	Dudzele	Dudzele	Dudzele	Melle
Number of animals at turn out	12	14	10	10	10	10
Average age at turn out (months)	7	4.5	9.5	5.5	10	9
Average body weight (kg)	222	164	264	155	221	242
Date of turn out	08/05/2013	10/05/2000	08/05/1996	08/05/1995	02/05/1994	10/05/1994
Date of stabling	14/10/2013	13/09/2000	23/10/1996	06/11/1995	10/10/1994	11/10/1994
Stocking rate (animals per hectare)	6-7	-	-	-	-	-
Anthelmintic treatment performed	No	No	No	No	Yes	No
Date(s) of anthelmintic treatment	-	21/08/2000	-	-	09/07/1994; 29/07/1994	-
Anthelmintic substance	-	Levamisole	-	-	Levamisole	-

Immunity-mediated regulation of the parasite population

Host acquired immunity is assumed to regulate the parasite population in 3 ways: 1) by exclusion of ingested larvae (increased pre-adult mortality rate), 2) by decreasing the survival of established (adult) nematodes and 3) by decreasing the fecundity of adult nematodes (Barger et al. 1985; Smith and Grenfell 1985; Coyne and Smith 1992; Smith 1994; Stear et al., 1995; Claerebout and Vercruysse, 2000). Thus immunity-mediated regulation of the parasite population was incorporated by increasing the mortality rates of pre-adult (μ_1; *equation 6*) and adult nematodes (μ_3; *equation 6*) and decreasing fecundity (λ; *equation 7*) with increasing acquired immunity. As acquired immunity cannot be measured directly (Claerebout and Vercruysse, 2000), little is known about the functional relationship between acquired immunity and these parameters. Therefore, a linear relationship was assumed, whereby mortality increases between the minimum and maximum values, and fecundity decreases between the maximum and minimum values as acquired immunity increases between 0 and 1:

$$\mu_i = \mu_{i(min)} + (\mu_{i(max)} - \mu_{i(min)})r \quad (6)$$

$$\lambda = \lambda_{(max)} - (\lambda_{(max)} - \lambda_{(min)})r \quad (7)$$

The mean and upper boundary of the 95% CI around the mean, estimated by meta-analysis, were used as minimum and maximum values for the pre-adult and adult mortality rates (Verschave et al., 2014, submitted). The lower boundary of the 95% CI around the mean and the mean, estimated by meta-analysis, were used as minimum and maximum values for the fecundity (Verschave et al., 2014, submitted).

Seasonally variable parameters

There is currently no consensus on the mechanisms of arrest and re-development in trichostrongylid nematodes and numerous confounding factors in available data prevent the development of robust mechanistic models of hypobiosis (Smith, 1974; Michel et al., 1976; Frank et al., 1986; 1988; Eysker, 1993; Fernández et al., 1999; Langrova and Jankovsk, 2004;

Lützelschwab et al., 2005; Langrova et al., 2008). As the numerous potential drivers of arrest are correlated and seasonal e.g. the age-structure of host populations, temperature, moisture and photoperiod, a simplified seasonal approach was taken to simulate seasonal variations in arrest rates, similar to that of Smith and Grenfell (1985). Arrest rate was assumed to vary with daylength (*d*; hours), whereby the minimum arrest rate coincided with the longest day and the maximum arrest rate coincided with the shortest day. Thus, arrest rate (*h*) at time *t* is a function of the minimum and maximum observed arrest rates, annual minimum and maximum daylength at the study site, and daylength at time *t* (equation 8).

$$h = h_{(max)} - \left(\frac{h_{(max)} - h_{(min)}}{d_{(max)} - d_{(min)}}\right) \times \left(d_t - d_{(min)}\right) \quad (8)$$

The lower and upper boundaries of the 95% CI around the average, estimated by meta-analysis, were used as minimum and maximum arrest rates (Verschave et al., 2014, submitted). Daylength can be estimated for any study site using the *daylength* function in the R package *geosphere* (Hijmans et al., 2012).

The proportion of arrested L4 resuming development was assumed to be an inverse function of daylength (equation 9):

$$\delta_2 = \left(\frac{1}{d_{(max)} - d_{(min)}}\right) \times \left(d_t - d_{(min)}\right) \quad (9)$$

Host grazing behaviour, dry matter intake and faecal production

To mimic a realistic infection risk, host-grazing behaviour in relation to the vertical distribution of L3 on herbage was taken into account. Pasture infectivity is usually expressed as the number of L3 per kilogram of dry herbage and is measured using the entire sward length. Grazing cattle, however, only ingest the top parts of the swards. When grazing behaviour is not taken into account, the infection rate is likely to be overestimated since most larvae are found close to the soil (Callinan and Westcott, 1986). To calculate the correction factor, a grazing height of 5 cm was assumed based on mean post-grazing grass heights of 5-6 cm reported by European dairy and cattle farmers and grazing experts (Phelan P., unpublished) and data on

the vertical distribution of L3 from various GIN species was used (Crofton, 1948; Silangwa and Todd, 1964; Callinan and Westcott, 1986). Finally, the measured pasture infectivity (L3 on herbage, *L3h*) was corrected with a factor of 0.14 (Appendix B). To calculate the daily number of L3 ingested the average daily dry matter intake (DMI) by grazing animals needs to be estimated. Cattle DMI was estimated based on bodyweight using the equations of MAFF (1975). The equations for growing young stock and adult cows were used for animals with a bodyweight of less than and more than 400kg, respectively, and the number of L3 ingested was estimated as follows:

$$L3i = DMI\ x\ 0.14L3h \qquad (10)$$

The average daily faecal production was estimated based on the host bodyweight as in Verschave et al. (2014, submitted). For this, the daily manure production was computed based on the formula of Nennich et al. (2005) using the animal bodyweight. The estimate was corrected for the average proportion of urine found in cattle manure (Verschave et al., 2014, submitted). Because no daily observations were available for the bodyweight, it was estimated starting from the bodyweight at turn out using standard age-related growth curves for dairy cattle (Cue et al., 2012).

3.2.3 Model validation

Collection of longitudinal parasitological data

Parasitological data from first season grazers on 7 commercial dairy farms in Flanders (Belgium) were collected during the grazing season of 2012 and 2013. The selected herds were visited monthly from turn out in Spring (April, May or June) until housing in Autumn (September, October or November). The average age at turn out varied between 6 and 21 months (Table 3.4).

Faecal egg counts of all animals were performed each month using a modified McMaster technique with a sensitivity of 10 eggs per gram faeces (epg) (MAFF, 1986). For nematode species identification, the positive faecal samples were mixed per herd and cultured according to Borgsteede and Hendriks (1973). Pasture infectivity (*L3h*) was measured, as described in

Verschave et al. (2015), each month and every two months respectively in 2012 and 2013 using the modified technique of Taylor (1939). At the start, the middle and the end of the pasture season animals were also weighed. The data collected from each herd formed a separate validation dataset.

Model validation - immune response rate

The collected field observations were used to compare and validate model predictions of the average FEC for each group of first season grazing cattle for models parameterised using the estimated and fitted response rates. Daily pasture contamination, dry matter intake and faeces production were estimated as described above. The goodness of fit between model predictions and observations was then assessed using a linear regression through the origin of observed and predicted FECs, as described by Rose et al. (2015). A perfect linear fit between model predictions and field observations implies an intercept of zero and a slope of 1. A regression through the origin with a slope that is not significantly different from 1 and therefore included in the 95% CI indicates a good fit. Competing models are evaluated based on the statistical significance of the regression through the origin, R^2 (higher is better), error (residual sum of squares; lower is better) and whether the slope is significantly different from 1. Due to the relatively small number of individuals in each herd, the potential for considerable individual variation in FECs (Levecke et al., 2011), and the limitations of the McMaster's faecal egg counting method (Morgan, E. R. unpublished data), a higher significance level of 0.1 was adopted and visual comparison of observed and predicted values were incorporated into the evaluation to mitigate against this variability undermining statistical validation.

Table 3.4 Characteristics of the first season grazing stock and the grazing season used for the collection of longitudinal parasitological data. These data were used for model validation. Approximate stocking rates are shown where the values are known.

	Herd 1	**Herd 2**	**Herd 3**	**Herd 4**	**Herd 5**	**Herd 6**	**Herd 7**
Location	Dudzele	Malle	Evergem	Oudenaarde	Drongen	Sinaai	Eeklo
Number of animals at turn out	11	12	10	19	11	16	16
Average age at turn out (months)	20	6	21	19	11	15	10
Average body weight (kg)	487	99	439	505	361	375	264
Date of turn out	20/04/2012	15/05/2012	14/06/2013	20/04/2013	07/06/2013	20/06/2013	13/06/2013
Date of stabling	09/11/2012 *	06/09/2012	20/09/2013 *	26/11/2013 *	14/10/2013	30/11/2013	10/09/2013
Stocking rate (animals per hectare)	-	-	5.2*	13*	62.4	3.7	25.5
Anthelmintic treatment performed	Yes	Yes	No	No	No	No	Yes
Date of anthelmintic treatment	7/9/2012	7/9/2012	-	-	-	-	19/08/2013
Anthelmintic substance	Moxidectin, pour-on formulation	Doramectin, injectable formulation	-	-	-	-	Moxidectin, pour-on formulation

* Several animals of these herds were stabled earlier due to impending partus.

Model validation – grazing behaviour

In a second round of validation both the longitudinal field observations and additional field trial data (Tables 3.3 and 3.4) were used to validate the model parameterised using only the estimated immune response rate to compare simulations incorporating grazing behaviour with those not incorporating grazing behaviour. As the additional field trial data were not used for the estimation of the immune response they are therefore independent datasets. Simulation and statistical validation methods were as described above.

3.3 Results

3.3.1 Model validation - immune response rate

Simulations using the estimated and fitted immune response rates for *O. ostertagi* and *C. oncophora* reproduced general observed patterns of FECs over the course of a grazing season in first season grazers (Figures 3.2 and 3.3; Table 3.5). The model parameterised using the estimate response rate resulted in systematically higher predicted FECs, compared to the model parameterised using the fitted response rate (Figures 3.2 and 3.3). However, there was very little practical difference in the predicted FECs nor in the statistical validation of both models. Therefore, the immune response rate estimated from the data of Shaw et al. (1998) was used henceforth.

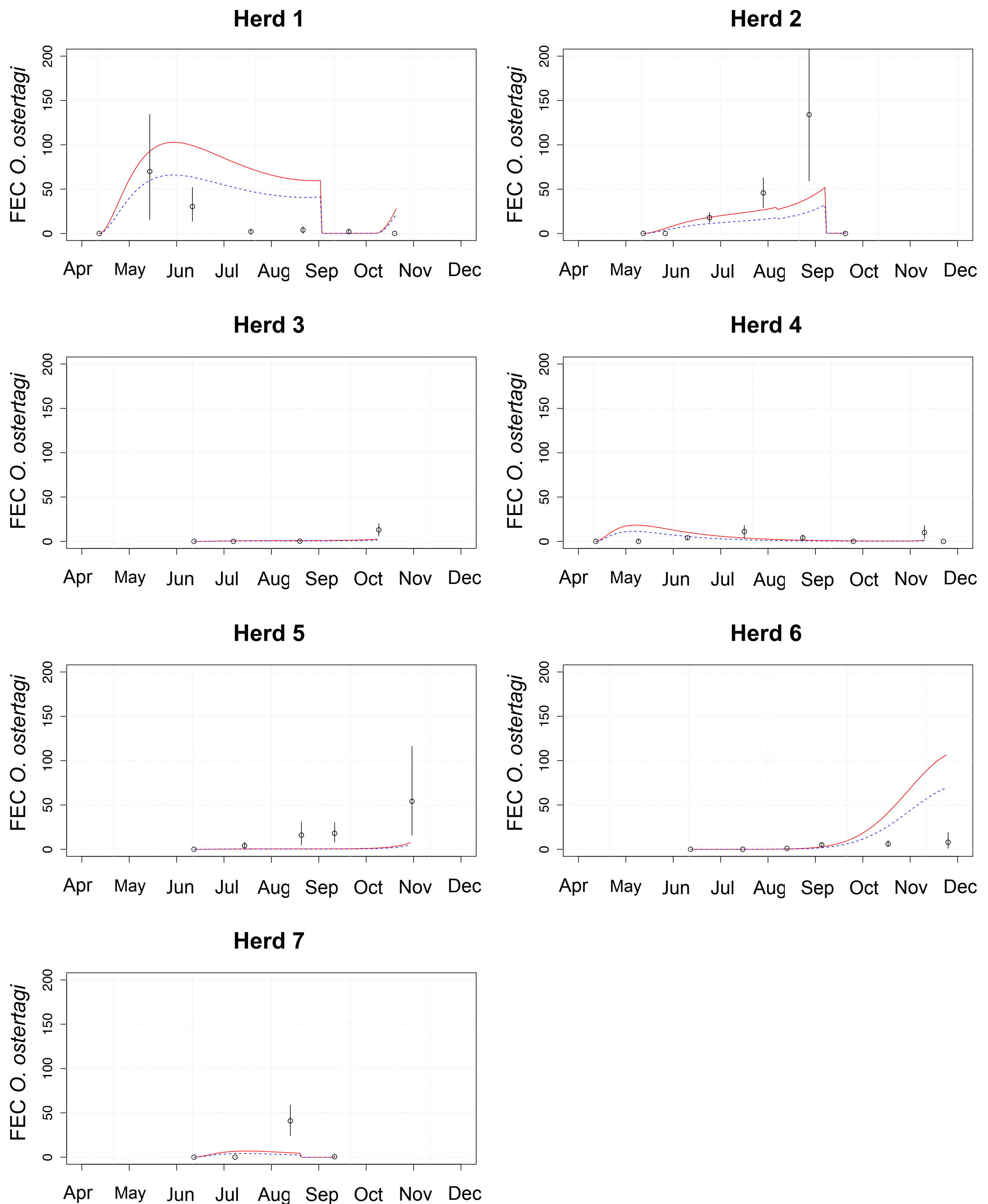


Figure 3.2. Observed and predicted faecal egg counts (FEC) for *O. ostertagi* of first season grazing animals of seven commercial dairy herds in Belgium. Animals were followed for the entire length of the first grazing season, further information on the background of this data can be found in Table 3.4. Points and error bars show the observed number of eggs per gram faeces (epg) and the corresponding 95% CI obtained by bootstrapping. The red full and blue dotted lines depict predictions obtained using the model in which the parameter for the level of acquired immunity was estimated and fitted respectively.

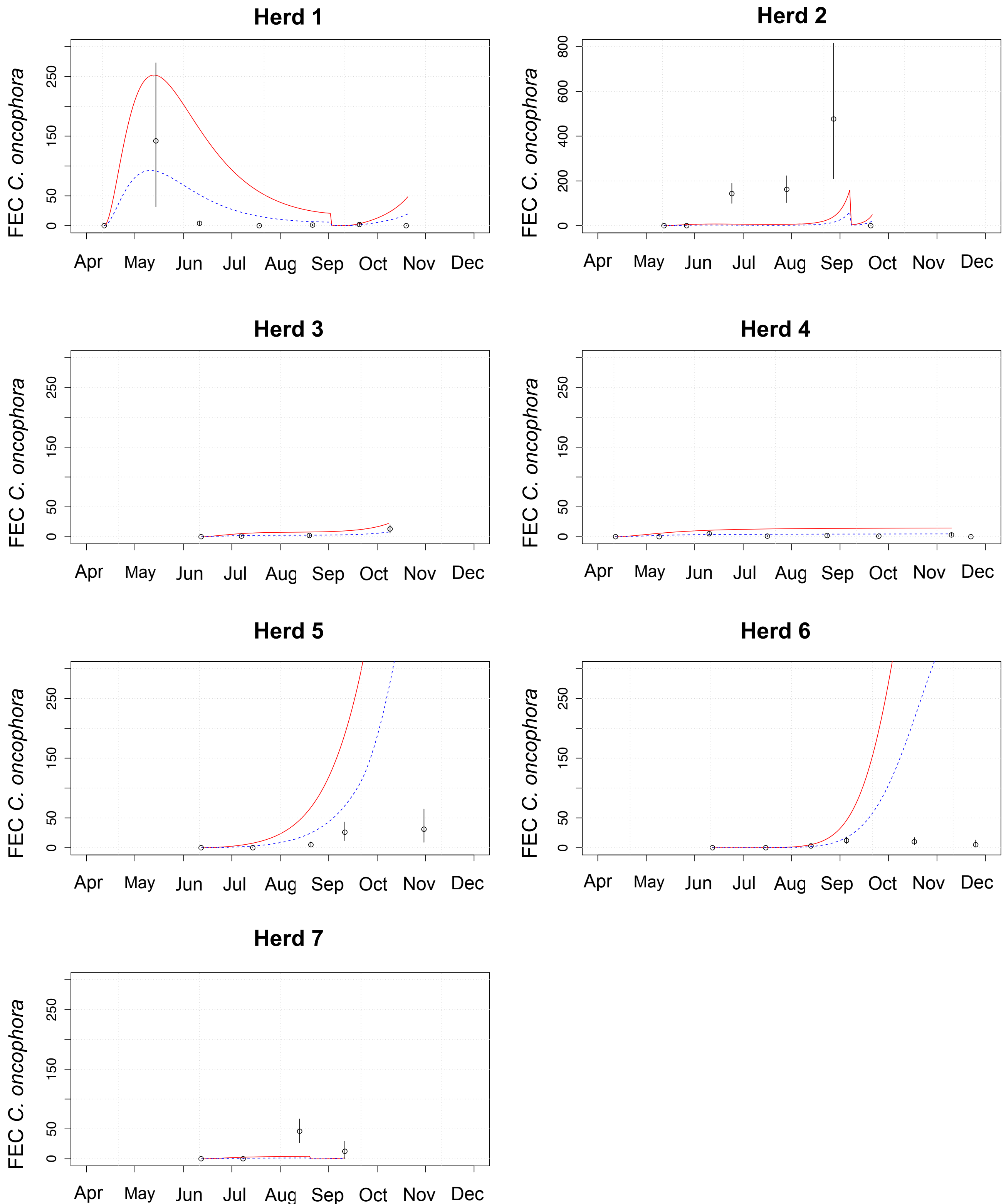


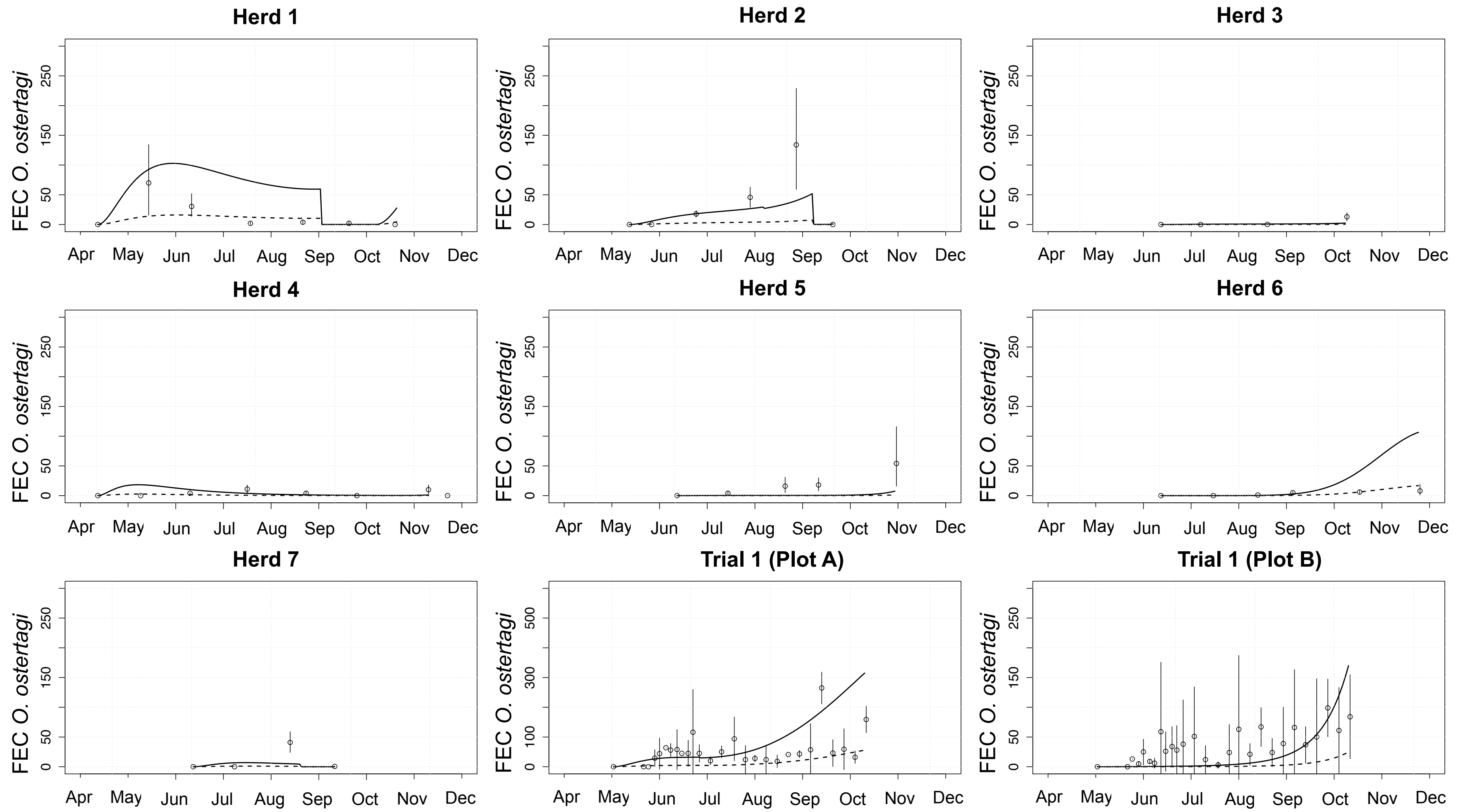
Figure 3.3. Observed and predicted faecal egg counts (FEC) for *C. oncophora* of first season grazing animals of seven commercial dairy herds in Belgium. Animals were followed for the entire length of the first grazing season, further information on the background of this data can be found in Table 3.4. Points and error bars show the observed number of eggs per gram faeces (epg) and the corresponding 95% CI obtained by bootstrapping. The red full and blue dotted lines depict predictions obtained using the model in which the parameter for the level of acquired immunity was estimated and fitted respectively.

Table 3.5. Validation of simulations for faecal egg counts (FEC) of *O. ostertagi* and *C. oncophora* using parasitological data of first season grazing animals of seven commercial dairy herds in Belgium. Different model versions were compared to assess the parameterisation of the immune response (estimated from data, or fitted to data). Statistically significant regressions with a critical p value of <0.1 and acceptable slopes (95% confidence interval spans 1) are highlighted in bold.

		Ostertagia ostertagi				***Cooperia oncophora***			
Dataset	**Parameter-isation of acquired immunity**	**Error (residual sum of squares)**	**Linear regression**	**R^2 (R^2 adjusted)**	**Slope (95% CI)**	**Error (residual sum of squares)**	**Linear regression**	**R^2 (R^2 adjusted)**	**Slope (95% CI)**
Herd 1	Estimated	43.27	$F_{1,6}$ = 8.92, p=0.024	0.60 (0.53)	1.69 (0.57 – 2.81)	70.31	$F_{1,6}$ = 13.35, p= 0.011	0.68 (0.64)	1.81 (0.85 – 2.77)
	Fitted	28.72	$F_{1,6}$ = 8.41; p = 0.027	0.58 (0.51)	1.09 (0.35 – 1.83)	22.50	$F_{1,6}$ = 17.05, p= 0.006	0.74 (0.70)	0.65 (0.34 – 0.96)
Herd 2	Estimated	8.18	$F_{1,4}$ = 35.29, p = 0.002	0.88 (0.85)	0.34 (0.22 – 0.46)	18.28	$F_{1,4}$ = 5.00, p = 0.076	0.50 (0.40)	0.08 (0.02 – 0.14)
	Fitted	4.93	$F_{1,4}$ = 36.11, p = 0.002	0.88 (0.85)	0.21 (0.15 – 0.27)	7.79	$F_{1,4}$ = 3.82, p = 0.108	0.43 (0.32)	0.03 (0.01 – 0.05)
Herd 3	Estimated	0.30	$F_{1,2}$ = 9.97, p = 0.087	0.83 (0.75)	3.90 (1.47 – 6.33)	0.83	$F_{1,2}$ = 126.30, p = 0.008	0.98 (0.98)	4.18 (3.45 – 4.90)
	Fitted	0.19	$F_{1,2}$ = 8.23, p = 0.103	0.80 (0.71)	2.27 (0.72 – 3.82)	0.41	$F_{1,2}$ = 57.20, p = 0.017	0.97 (0.95)	1.40 (1.04 – 1.76)
Herd 4	Estimated	8.35	$F_{1,6}$ = 0.56, p = 0.48	0.09 (-0.06)	0.39 (-0.63 – 1.41)	7.55	$F_{1,6}$ = 10.24, p = 0.019	0.63 (0.57)	3.82 (1.48 – 6.16)
	Fitted	5.01	$F_{1,6}$ = 0.41, p = 0.54	0.06 (-0.09)	0.20 (-0.41 – 0.82)	2.41	$F_{1,6}$ = 10.60, p = 0.017	0.64 (0.58)	1.24 (0.49 – 1.99)
Herd 5	Estimated	0.13	$F_{1,3}$ = 22.61, p = 0.018	0.88 (0.84)	0.03 (0.02 – 0.04)	18.16	$F_{1,3}$ =120.90, p = 0.002	0.98 (0.97)	7.54 (6.20 – 8.88)
	Fitted	0.08	$F_{1,3}$ = 19.01, p = 0.022	0.86 (0.82)	0.02 (0.01 – 0.03)	6.57	$F_{1,3}$ =126.00, p = 0.002	0.98 (0.97)	2.79 (2.30 – 3.28)
Herd 6	Estimated	12.32	$F_{1,4}$ = 7.32, p = 0.054	0.65 (0.56)	4.23 (1.17 – 7.29)	199.90	$F_{1,4}$ =3.65, p = 0.129	0.48(0.35)	24.01 (-0.63 – 48.65)
	Fitted	7.79	$F_{1,4}$ = 7.33, p = 0.054	0.65 (0.56)	2.68 (0.74– 4.62)	78.69	$F_{1,4}$ =3.61, p = 0.130	0.47 (0.34)	9.39 (-0.31 – 19.09)
Herd 7	Estimated	3.83	$F_{1,3}$ = 1.33, p = 0.332	0.36 (0.15)	0.12 (-0.06 – 0.30)	1.48	$F_{1,3}$=8.36,p = 0.063	0.74 (0.65)	0.09 (0.03 – 0.15)
	Fitted	2.38	$F_{1,3}$ = 1.33, p = 0.332	0.31 (0.08)	0.07 (-0.05 – 0.19)	0.55	$F_{1,3}$= 6.79, p = 0.080	0.69 (0.59)	0.03 (0.01 – 0.05)

3.3.2 Model validation – grazing behaviour

Simulations were successful in replicating patterns of FECs over the grazing season (Figures 3.4 and 3.5) and a statistically significant regression through the origin was achieved at the 0.1 significance level for 32/36 (*O. ostertagi*) and 26/36 (*C. oncophora*) of the evaluations. Of these, 28 (*O. ostertagi*) and 21 (*C. oncophora*) achieved significance at the 0.05 significance level (Table 3.6). There were significant deviations in the slope from 1 (Table 3.6) indicating systematic under- or over-prediction. Models incorporating grazing behaviour tended to underestimate FECs (slope significantly lower than 1) whereas simulations not incorporating grazing behaviour tended to overestimate FECs. Simulations incorporating grazing behaviour always minimized error. For a number of validation datasets this was due to the tendency to overestimate FEC towards the end of the grazing season (e.g. Herd 6, Figure 3.4), and was particularly pronounced for *C. oncophora* (Figure 3.5). Overall, R^2 values were similar for models incorporating grazing behaviour and not incorporating grazing behaviour. Therefore, based on the statistical evaluation, overall, models incorporating grazing behaviour appeared to outperform those not incorporating grazing behaviour. However, qualitative evaluation of the simulation output against observed FECs shows that model performance varied by herd/trial. For some herds and trials simulations using models not incorporating grazing behaviour appeared to have a better fit; e.g. Trial 1 (plot E) (*O. ostertagi*), Trial 1 (plot F) *(O. ostertagi*), Trial 6 (*O. ostertagi*) and Herd 2 (*O. ostertagi* and *C. oncophora*).



Herd 1
Herd 2
Herd 3
Herd 4
Herd 5
Herd 6
Herd 7
Trial 1 (Plot A)
Trial 1 (Plot B)
FEC O. ostertagi
Apr May Jun Jul Aug Sep Oct Nov Dec
0 50 150 250
0 100 300 500

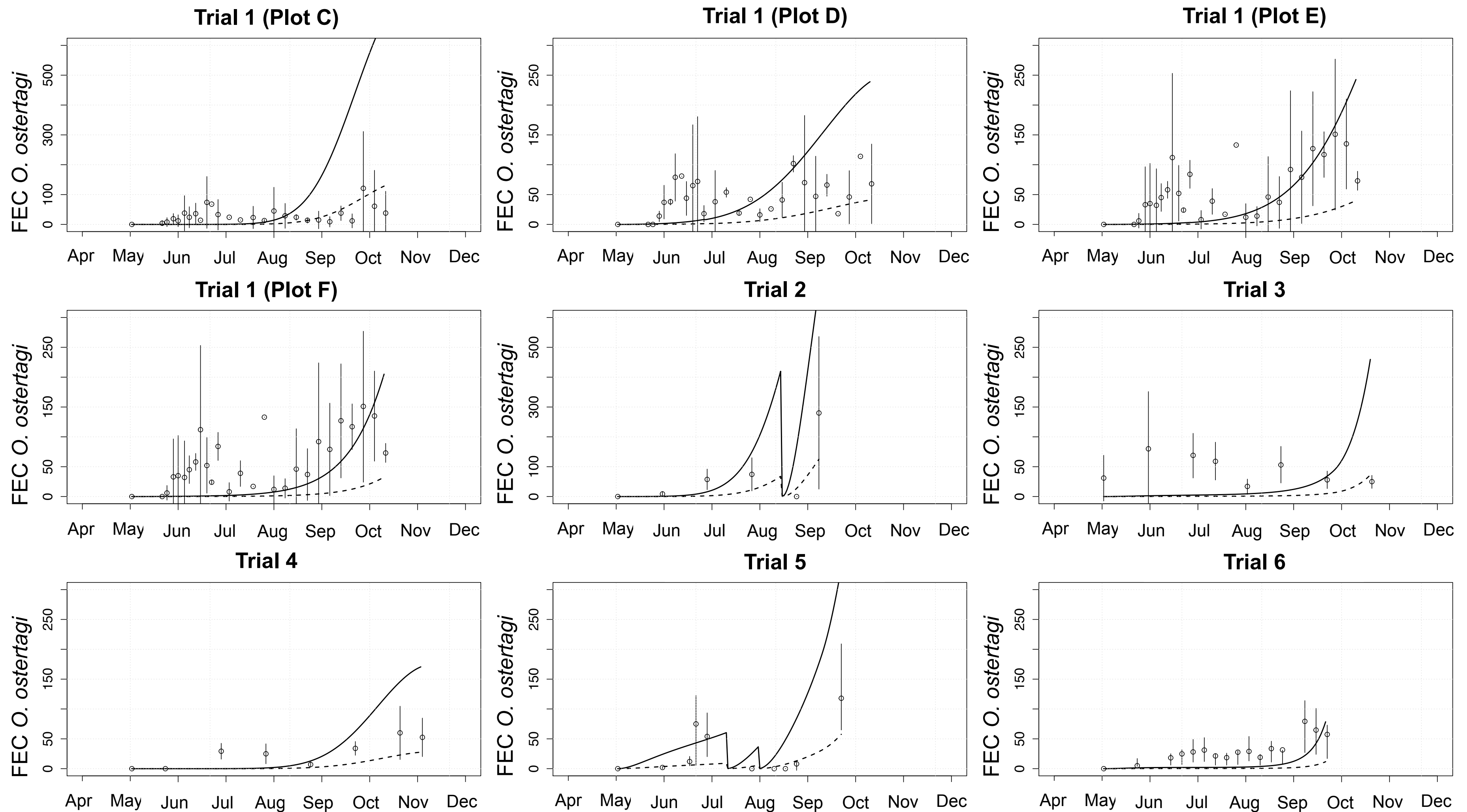
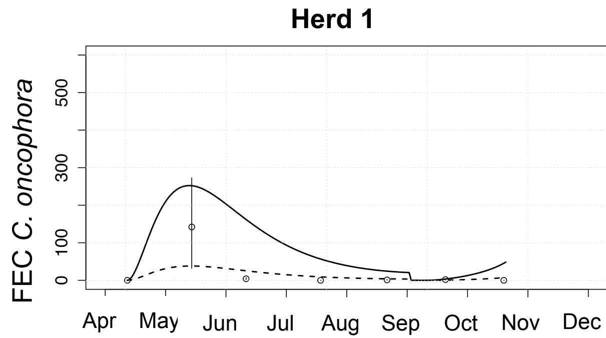
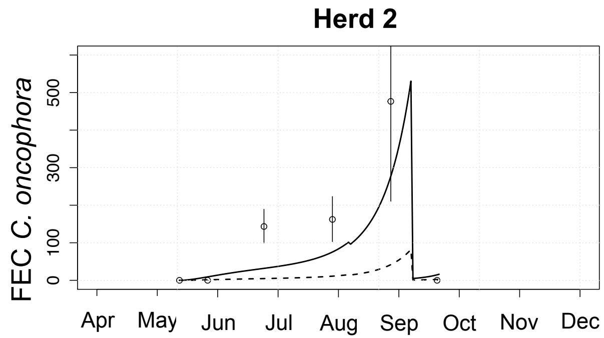
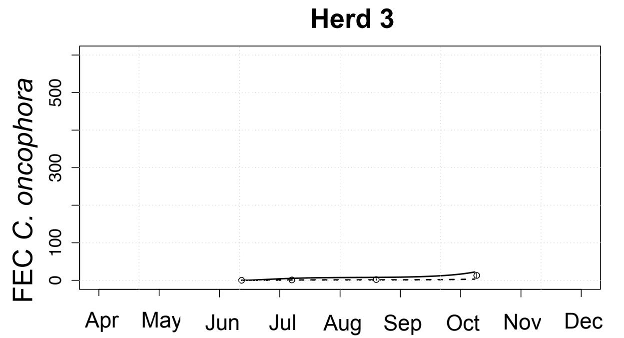
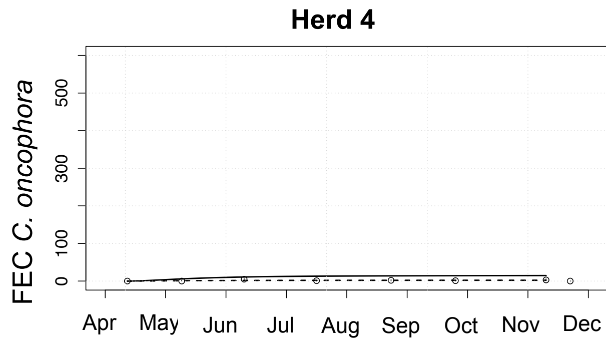


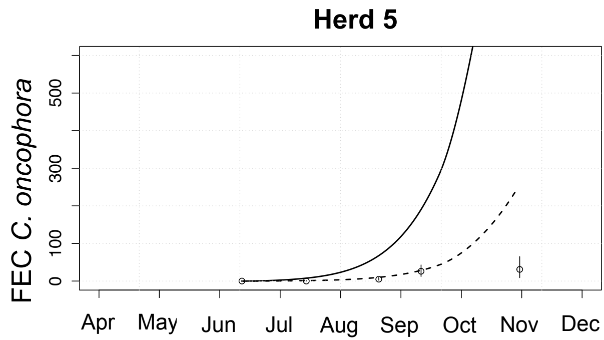
Figure 3.4. Observed and predicted faecal egg counts (FEC) for *O. ostertagi* of first season grazing animals of seven commercial dairy herds in Belgium. Animals were followed for the entire length of the first grazing season, further information on the background of this data can be found in Tables 3.3 and 3.4. Points and error bars show the observed number of eggs per gram faeces (epg) and the corresponding 95% confidence interval obtained by bootstrapping. The solid lines depict predictions obtained using the model in which the grazing behaviour was not incorporated, the dashed lines in which the grazing behaviour was incorporated.

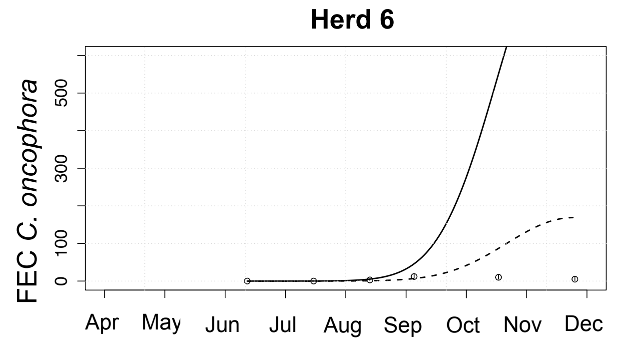

Herd 1
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

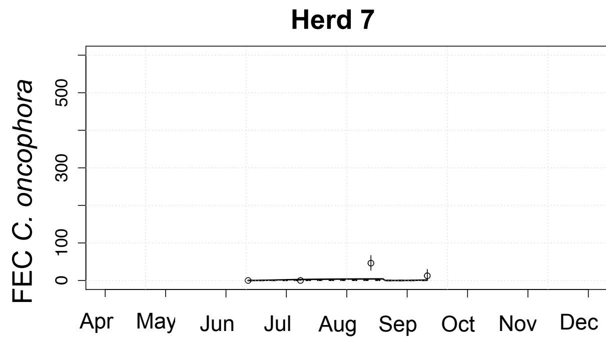

Herd 2
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

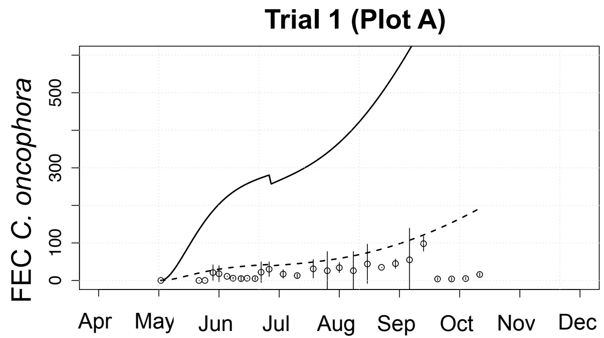

Herd 3
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

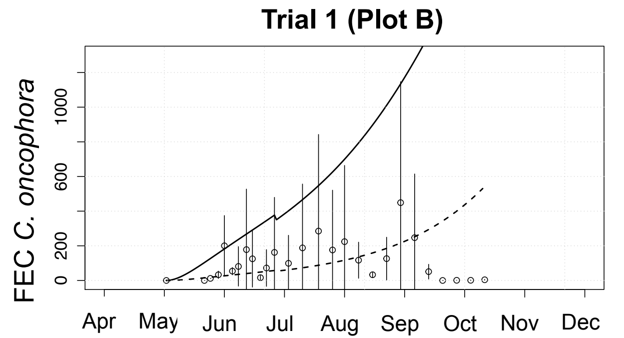

Herd 4
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


Herd 5
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


Herd 6
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


Herd 7
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


Trial 1 (Plot A)
FEC C. oncophora
0
100
300
500
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


Trial 1 (Plot B)
FEC C. oncophora
0
200
600
1000
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

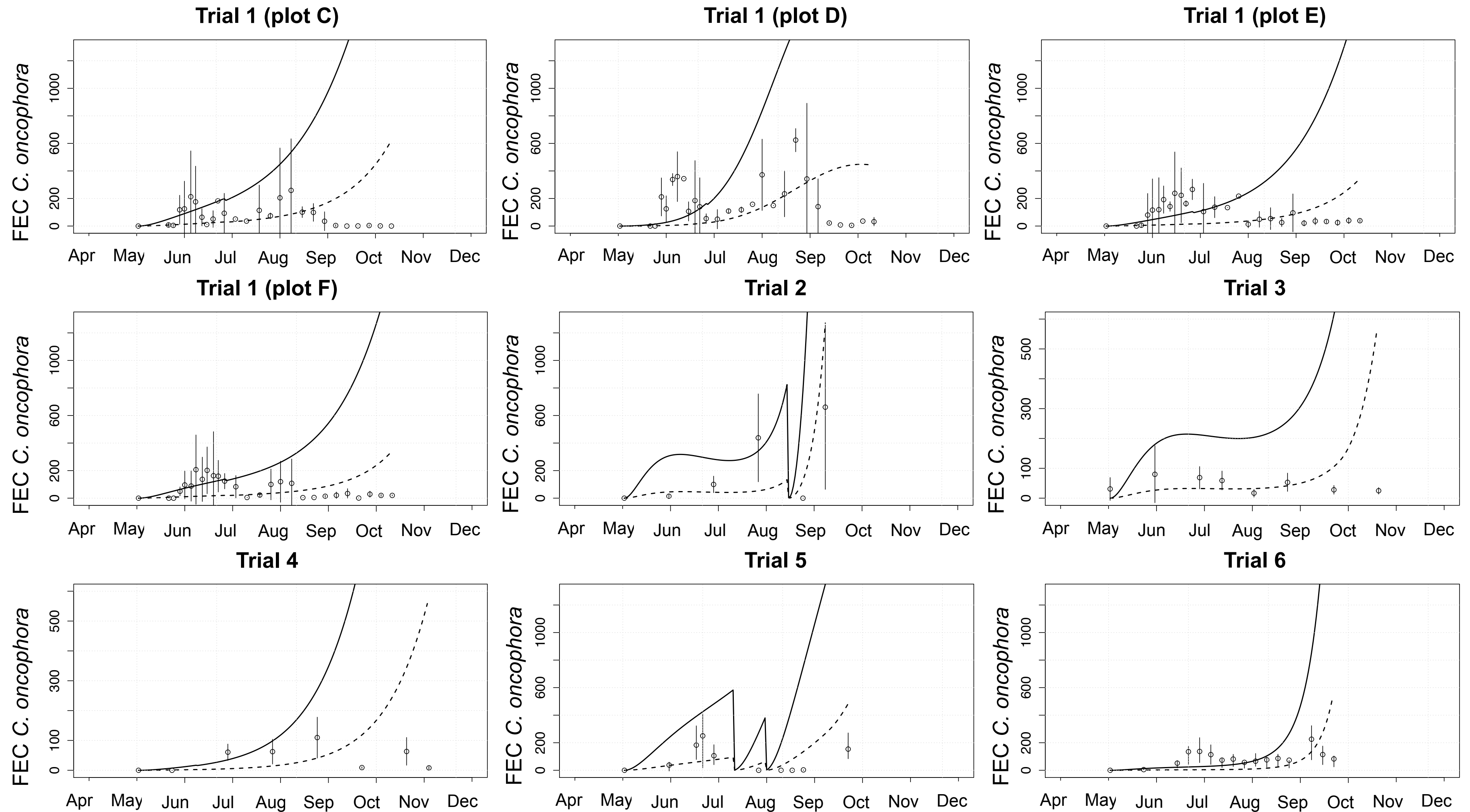


Figure 3.5. Observed and predicted faecal egg counts (FEC) for *C. oncophora* of first season grazing animals of seven commercial dairy herds in Belgium. Animals were followed for the entire length of the first grazing season, further information on the background of this data can be found in Tables 3.3 and 3.4. Points and error bars show the observed number of eggs per gram faeces (epg) and the corresponding 95% confidence interval obtained by bootstrapping. The solid lines depict predictions obtained using the model in which the grazing behaviour was not incorporated, the dashed lines in which the grazing behaviour was incorporated.

Table 3.6. Validation of simulations for faecal egg counts (FEC) of *O. ostertagi* and *C. oncophora* using parasitological data of first season grazing animals on seven commercial dairy herds in Belgium (Table 3.4) and additional field trials (Table 3.3). The model was parameterised using the estimated immune response rate and different model versions were compared to evaluate the impact of using a correction factor to incorporate grazing behaviour of cattle and the vertical distribution of nematodes on herbage.

		Ostertagia ostertagi				***Cooperia oncophora***			
Dataset	**Grazing behaviour**	**Error (residual sum of squares)**	**Linear regression**	R^2 (R^2 adjusted)	**Slope (95% CI)**	**Error (residual sum of squares)**	**Linear regression**	R^2 (R^2 adjusted)	**Slope (95% CI)**
Herd 1	No	43.27	$F_{1,6}$ =8.92, p=0.024	0.60 (0.53)	1.69 (0.57 – 2.81)	70.31	$F_{1,6}$ =13.35, p=0.011	0.68 (0.64)	1.81 (0.85 – 2.77)
	Yes	7.36	$F_{1,6}$ =7.39; p=0.035	0.55 (0.47)	0.26 (0.06 – 0.46)	11.09	$F_{1,6}$ =12.32, p=0.013	0.67 (0.62)	0.27 (0.11 – 0.43)
Herd 2	No	8.18	$F_{1,5}$ =35.29, p=0.002	0.88 (0.85)	0.34 (0.22 – 0.46)	24.13	$F_{1,5}$ =138.90, p<0.001	0.97 (0.96)	0.54 (0.45 – 0.63)
	Yes	1.19	$F_{1,5}$ =37.38, p=0.002	0.88 (0.86)	0.05 (0.03 – 0.07)	3.73	$F_{1,5}$ =131.40, p<0.001	0.96 (0.96)	0.08 (0.07 – 0.10)
Herd 3	No	0.30	$F_{1,2}$ =9.97, p=0.087	0.83 (0.75)	3.90 (1.47 – 6.33)	0.83	$F_{1,2}$ =126.30, p=0.008	0.98 (0.98)	4.18 (3.45 – 4.90)
	Yes	0.04	$F_{1,2}$ =9.99, p=0.087	0.83 (0.75)	0.57 (0.12 – 1.02)	0.12	$F_{1,2}$ =128.00, p=0.008	0.98 (0.98)	0.61 (0.51 – 0.71)
Herd 4	No	8.35	$F_{1,6}$ =0.56, p=0.48	0.09 (-0.06)	0.39 (-0.63 – 1.41)	7.55	$F_{1,6}$ =10.24, p=0.019	0.63 (0.57)	3.82 (1.48 – 6.16)
	Yes	1.23	$F_{1,6}$ =0.58, p=0.48	0.09 (-0.06)	0.06 (-0.09 – 0.21)	1.11	$F_{1,6}$ =10.20, p=0.019	0.63 (0.57)	0.56 (0.21 – 0.91)
Herd 5	No	0.13	$F_{1,3}$ =22.61, p=0.018	0.88 (0.84)	0.03 (0.02 – 0.04)	18.16	$F_{1,3}$ =120.90, p=0.002	0.98 (0.97)	7.54 (6.20 – 8.88)

	Yes	0.02	$F_{1,3}$ =22.62, p=0.018	0.88 (0.84)	0.01 (0.00 – 0.01)	2.61	$F_{1,3}$ =129.70, p=0.001	0.98 (0.97)	1.12 (0.92 – 1.32)
Herd 6	No	12.32	$F_{1,4}$ =7.32, p=0.054	0.65 (0.56)	4.23 (1.17 – 7.29)	199.90	$F_{1,4}$ =3.65, p=0.129	0.48(0.35)	24.01 (-0.63–48.65)
	Yes	1.83	$F_{1,4}$ =7.28, P=0.054	0.65 (0.56)	0.63 (0.18 – 1.08)	31.67	$F_{1,4}$ =3.56, p=0.132	0.47 (0.34)	3.76 (-0.14 – 7.66)
Herd 7	No	3.83	$F_{1,3}$ =1.33, p=0.332	0.36 (0.15)	0.12 (-0.06 – 0.30)	1.48	$F_{1,3}$=8.36, p=0.063	0.74 (0.65)	0.09 (0.03 – 0.15)
	Yes	0.56	$F_{1,3}$ =1.70, p=0.283	0.36 (0.15)	0.02 (0.00 – 0.04)	0.22	$F_{1,3}$=8.37, p=0.063	0.74 (0.65)	0.01 (0.00 – 0.02)
Trial 1 (plot A)	No	85.43	$F_{1,25}$ =17.80, p<0.001	0.42 (0.39)	1.00 (0.53 – 1.46)	297.50	$F_{1,25}$ =29.93, p<0.001	0.55 (0.53)	10.47 (6.72–14.21)
	Yes	14.47	$F_{1,25}$ =16.16, p<0.001	0.39 (0.37)	0.16 (0.08 – 0.24)	54.50	$F_{1,25}$ =25.30, p<0.001	0.50 (0.48)	1.76 (1.08 – 2.45)
Trial 1 (plot B)	No	34.99	$F_{1,25}$ =22.50, p<0.001	0.47 (0.45)	0.79 (0.46 – 1.11)	717.70	$F_{1,25}$ =11.63, p=0.002	0.32 (0.29)	3.08 (1.31 – 4.84)
	Yes	5.42	$F_{1,25}$ =21.70, p<0.001	0.46 (0.44)	0.12 (0.07 – 0.17)	159.70	$F_{1,25}$ =7.42, p=0.012	0.23 (0.20)	0.55 (0.15 – 0.94)
Trial 1 (plot C)	No	156.10	$F_{1,25}$ =15.85, p<0.001	0.39 (0.36)	3.08 (1.56 -4.60)	776.90	$F_{1,25}$ =2.21, p=0.149	0.08 (0.04)	2.08 (-0.66 – 4.83)
	Yes	27.08	$F_{1,25}$ =15.93, p<0.001	0.39 (0.36)	0.54 (0.27 – 0.80)	160.40	$F_{1,25}$ =1.32, p=0.261	0.05 (0.01)	0.33 (-0.23 – 0.90)
Trial 1 (plot D)	No	67.61	$F_{1,25}$ =20.85, p<0.001	0.45 (0.43)	1.14 (0.65 – 1.62)	862.50	$F_{1,25}$ =8.92, p=0.006	0.26 (0.23)	2.27 (0.78 – 3.76)
	Yes	11.02	$F_{1,25}$ =20.05, p<0.001	0.45 (0.42)	0.18 (0.10 – 0.62)	193.80	$F_{1,25}$ =5.94, p=0.022	0.19 (0.16)	0.42 (0.08 – 0.75)
Trial 1	No	40.49	$F_{1,25}$ =43.55,	0.64 (0.62)	0.71 (0.50 – 0.92)	474.50	$F_{1,25}$ =2.44,	0.09 (0.05)	1.16 (-0.29 – 2.61)

(plot E)			p<0.001				p=0.131		
	Yes	6.44	$F_{1,25}$=41.88, p<0.001	0.63 (0.61)	0.11 (0.08 – 0.14)	85.44	$F_{1,25}$=1.95, p=0.175	0.07 (0.04)	0.19 (-0.08 – 0.45)
Trial 1 (plot F)	No	29.52	$F_{1,25}$=34.25, p<0.001	0.58 (0.56)	0.46 (0.31 -0.62)	467.80	$F_{1,25}$=2.30, p=0.142	0.08 (0.05)	1.46 (-0.43 – 3.35)
	Yes	4.57	$F_{1,25}$=33.14, p<0.001	0.57 (0.55)	0.07 (0.05 – 0.09)	84.36	$F_{1,25}$=1.79, p=0.194	0.07 (0.03)	0.23 (-0.11 – 0.57)
Trial 2	No	94.45	$F_{1,5}$=55.60, p<0.001	0.92 (0.90)	2.38 (1.76 – 3.01)	1318.00	$F_{1,5}$=13.63, p=0.014	0.73 (0.68)	6.09 (2.86 – 9.32)
	Yes	16.51	$F_{1,5}$=56.90, p<0.001	0.92 (0.90)	0.42 (0.31 – 0.53)	303.10	$F_{1,5}$=12.85, p=0.016	0.72 (0.66)	1.36 (0.62 – 2.10)
Trial 3	No	9.38	$F_{1,6}$=8.64, p=0.026	0.59 (0.52)	0.31 (0.10 – 0.52)	228.20	$F_{1,6}$=5.59, p=0.056	0.48 (0.40)	3.87 (0.66 – 7.07)
	Yes	1.39	$F_{1,6}$=8.59, p=0.026	0.59 (0.52)	0.05 (0.02 – 0.08)	39.50	$F_{1,6}$=4.68, p=0.074	0.44 (0.34)	0.61 (0.06 – 1.17)
Trial 4	No	31.82	$F_{1,6}$=19.01, p=0.005	0.76 (0.72)	1.75 (0.96 – 2.54)	658.80	$F_{1,6}$=2.30, p=0.180	0.28 (0.16)	6.49 (-1.90 -14.87)
	Yes	5.00	$F_{1,6}$=18.62, p=0.005	0.76 (0.72)	0.27 (0.15 - 0.40)	129.30	$F_{1,6}$=2.06, p=0.201	0.26 (0.13)	1.20 (-0.44 – 2.85)
Trial 5	No	61.04	$F_{1,9}$=25.89, p<0.001	0.74 (0.71)	2.06 (1.27 -2.86)	636.00	$F_{1,9}$=6.05, p=0.036	0.40 (0.34)	4.30 (0.87 – 7.73)
	Yes	10.48	$F_{1,9}$=24.77, p<0.001	0.73 (0.70)	0.35 (0.21 – 0.48)	142.40	$F_{1,9}$=4.65, p=0.059	0.34 (0.27)	0.84 (0.08 - 1.61)
Trial 6	No	5.59	$F_{1,14}$=10.07, p=0.007	0.42 (0.38)	0.13 (0.05 – 0.21)	391.30	$F_{1,14}$=8.23, p=0.012	0.37 (0.33)	2.85 (0.90 - 4.80)
	Yes	0.82	$F_{1,14}$=10.03, p=0.007	0.42 (0.38)	0.02 (0.01 – 0.03)	66.96	$F_{1,14}$=7.43, p=0.016	0.35 (0.30)	0.46 (0.13 – 0.80)

3.4 Discussion

GLOWORM-PARA provides a generic model framework for the parasitic phase of GIN infections that can be adapted to different nematode species. The framework was parameterised and validated for two economically important nematode species of cattle, i.e. *O. ostertagi* and *C. oncophora*. To our knowledge, no previous attempt has been made to model *C. oncophora* transmission alone. For *O. ostertagi,* GLOWORM-PARA incorporates important improvements to the existing models such as parameterisation of the rate of acquisition of immunity based on cumulative exposure and the incorporation of host grazing behaviour. Both the parameterisation and validation of these models were supported by extensive datasets obtained from various sources and acquired over decades of parasitological research. This represents the most comprehensive and thorough validation of GIN models in ruminants to date.

The model framework replicated seasonal patterns of *O. ostertagi* and *C. oncophora* FECs in cattle during their first grazing season, ranging in age from 4.5 to 21 months at turnout. Faecal avoidance behaviour has been previously shown to be an important driver of transmission for GINs (Fox et al., 2013) due to heterogeneity in the spatial distribution of L3 on pasture. However, another relatively overlooked aspect of grazing behaviour is grazing height and its impact on predicted larvae intake rates. Here, two scenarios were compared. The first made no assumptions regarding grazing behaviour, effectively assuming that larvae were evenly distributed on pasture and on the herbage swards. The second incorporated mean grazing heights of cattle based on post-grazing herbage heights reported by European farmers and grazing experts (Phelan, P., unpublished data) and the mean proportion of L3 expected above this height based on data reported in the literature, to apply a correction factor to the larvae ingestion rate. Overall, incorporating grazing behaviour into simulations improved predictions by reducing error. However, this varied by herd and in a minority of herds simulations not incorporating grazing behaviour appeared to be a better fit to the data. Data on stocking rates and available biomass were not available for all herds and trials used for validation in this study but may account for this variability. This should be

considered when applying this model framework to different management systems. For example, cattle that are strip grazed may be more likely to graze to a lower height than those that are set stocked immediately after turnout, when biomass is high. Furthermore, grazing height and the density of L3 on pasture will vary in set-stocked herds throughout the grazing season as grass growth varies. The simulations presented here assumed constant biomass throughout the grazing season due to the lack of data and models to track grass growth. However, incorporating grass growth may improve predictions if adequate predictive models become available.

The development of mechanistic models is often impaired by an incomplete knowledge of the system to be modelled and the lack of process-oriented data is still one of the biggest challenges involved in modelling parasitic lifecycles (Sutherst, 2001; Morgan, 2013). Acquired immunity is known to regulate establishment, survival and fecundity of GIN, which makes it a crucial driver of the population dynamics during the parasitic phase. However, direct quantification of acquired immunity proves to be difficult. Different mechanisms probably underlie the different effects of immunity (Kloosterman et al., 1978; Stear et al., 1995) and also differences between nematode species need to be considered (Armour, 1989). Some existing models e.g. Singleton et al. (2011) use an immune response based on observed antibody titres. Although similar data were available for *O. ostertagi* and *C. oncophora*, matching antibody titres to a level of acquired immunity is difficult as the precise mechanisms underlying immunity to GINs are poorly defined despite extensive research (Claerebout and Vercruysse, 2000). Other existing models e.g. Grenfell et al. (1987a) vary immune-dependent traits such as fecundity with duration of infection. Although this method has been used to successfully reproduce seasonal patterns of infection, it would not be robust to simulations where the pasture infectivity and therefore exposure to infection is different to the scenario used to fit the model parameters. The development of acquired immunity, as defined and parameterised in GLOWORM-PARA, enables the comparison of scenarios in which significant changes in exposure to GINs and subsequently changes in the development of acquired immunity are expected. For example, climate-driven changes may result in changes to the seasonal

dynamics of pasture infectivity (Rose et al., 2015) but the consequences for the host's response is unknown.

Acquired immunity is also modelled here as a separate component that affects the larval establishment, adult survival and female fecundity of the in-host nematode population. Previous models often did not incorporate acquired immunity as a separate component (Gettinby et al., 1979; Gettinby and Paton, 1981; Grenfell et al., 1987a; Chaparro et al., 2013). The model of Grenfell et al. (1987a), for example, based its description of the regulation of key life history traits on the analysis of experimental observations (Grenfell et al., 1987b; Smith et al. 1987). A framework for the parasitic phase that encompasses a separate entity for immunity, however, provides a high level of flexibility to incorporate additional complexity at nematode species level when needed, or as our understanding of the mechanisms underlying immunity increases.

Recent generic models that explicitly incorporate immunity as a model component, have implemented theoretically chosen values for the parameterisation of immunity rather than using empirical estimates (Cornell, 2005). The current study therefore compared two different approaches to parameterise the response rate of acquired immunity to *O. ostertagi* and *C. oncophora*: parameter estimation versus parameter fitting. Data fitting often results in the creation of a 'black box' in which the underlying mechanisms and relevant drivers are unknown. When the fitted response rate was implemented, the resulting FEC predictions were systematically lower since protective immunity was acquired very quickly. Moreover, simulations based on the fitted response rate showed an immunity-build up that would not be regarded as realistic based on the current knowledge. Protective immunity for *O. ostertagi*, for example, is typically acquired after 1.5 grazing seasons while simulations based on the fitted response rate show a much more rapid acquisition of immunity (Armour, 1989; Ploeger et al., 1994; Claerebout et al., 1997; Ravinet et al., 2014). Therefore, the approach in which the response rate is estimated is preferred and recommended for further implementation of the GLOWORM-PARA model.

The extensive model validation completed in this study revealed that FECs may be over-predicted late in the grazing season in some herds, possibly due

to uncertainty surrounding the acquisition of immunity and knock-on effects on life-history parameters such as fecundity. The immune response to *O. ostertagi* and *C. oncophora* differs in the fact that acquired immunity to *C. oncophora* develops more quickly and/or more strongly compared to *O. ostertagi* (Armour 1989). Also, for *C. oncophora* different reports on worm expulsion during infections exist, which would explain the fact that the largest deviations between observations and simulations are seen some time after the start of the infection. The observed time point at which this expulsion event occurs, however, varies between reports (Smith and Archibald, 1968; Kloosterman et al., 1991; Kanobana et al., 2001; 2002). Therefore, future inclusion of density-dependence and expulsion may be beneficial if suitable data become available.

An influence of host age on the ability to develop immunity has also been raised, however, existing evidence on this is rather limited and contradictory (Smith and Archibald, 1968; Armour, 1989; Kloosterman et al., 1991). Furthermore, no impact of age-related immunity was seen in the validation of simulations presented here, which used data from first season grazers up to 2 years old. Moreover, it seems that differences in the ability to develop an effective immune response even exist between animals of the same age category and that animals can be divided in different responder types (Kanobana et al., 2001; 2004). The use of individual based modelling provides a means of dealing with such differences in the host population (Fox et al., 2013) but the complexity of such models renders simulations cumbersome and computationally expensive. Mean-field models such as the framework presented here provide a much more tractable solution to evaluate contrasting management scenarios and to merge with existing climate-dependent models of the free-living stages.

To conclude, a generic framework to simulate the parasitic phase of GIN infections is presented here and is used to simulate *O. ostertagi* and *C. oncophora* infections. The model was successful in simulating infection patterns of first season grazers for these nematode species. Both parameterisation and validation were supported by an extended database of field observations. The model framework is flexible and allows future adjustments such as the incorporation of additional complexity in the

acquisition of immunity. Future research will link GLOWORM-PARA with models that simulate the free-living phase of GIN to obtain a full lifecycle framework for the evaluation of alternative control strategies.

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Chapter 4

Measuring larval nematode contamination on cattle pastures

Adapted from

Verschave S.H., Levecke B., Duchateau L., Vercruysse J., Charlier J., 2015. Measuring larval nematode contamination on cattle pastures: comparing two herbage sampling methods. Veterinary Parasitology 210, 159-166.

4.1 Introduction

GINs are an important threat to economic livestock farming worldwide (Charlier et al., 2014). Ruminants get infected with these parasitic nematodes by ingestion of the free-living infective larvae (L_3) during grazing. Since long, assessment of the pasture larval contamination with L_3 has been used to understand the population dynamics of the free-living stages in epidemiological studies and to evaluate the effect of anthelmintic treatment programmes (Rickard et al., 1991; Satrija and Nansen, 1996; Bauer et al., 1997; Gossellin et al. 1998; Sargison et al, 2012). Pasture larval counts (PLC) will be used in the field validation of nematode vaccination strategies and targeted control programmes onwards (Le Jambre et al., 2008; Bassetto et al., 2014). As a proxy for the parasite infection risk to which animals are exposed, PLC serve both as input parameter and validation tool for the development of predictive nematode transmission models (Ward, 2006; Gaba et al., 2012; Laurenson et al., 2012a; 2012b; Fox et al., 2013; Rose et al., 2015).

Different techniques have been used to measure pasture larval contamination (Bryan and Kerr, 1988), including the use of grazing animals fistulated at the oesophagus, necropsy of tracer animals and direct quantification of L_3 on herbage. The ethical and economical aspects of using fistulated or tracer animals (Cabaret et al., 1986; Bryan and Kerr, 1988), put important limitations on the application of these techniques. These limitations do not apply to the direct quantification of L_3 on herbage. However, this technique has other important drawbacks: it is labour intensive (Boag et al., 1989; Demeler, 2012) and considerable variation is often seen between repeated measurements (Boag et al., 1989; Couvillion, 1993).

The process of direct quantification of L_3 on herbage consist generally of three phases; (1) herbage collection, (2) processing and (3) L_3-species identification (Couvillion, 1993). Until now, research to improve and facilitate quantification of L_3 on herbage has mainly focused on the two latter phases. Repeatability, recovery rates and speed of the processing phase have been improved during recent years (Demeler et al., 2012; Cassida et al., 2012) and also progress on molecular identification of L_3 on pasture samples has been

made (Sweeny et al., 2012; Bisset et al., 2014). Despite these efforts, the herbage collection process still needs to be addressed to facilitate the use of PLC as routine diagnostic. Traditionally, herbage collection is done by walking a double-crossed W-transect across a pasture (Taylor et al., 1939). Throughout the years, modifications on this method have been made (e.g. Lancaster, 1970; Bryan and Kerr, 1988; Aumont and Gruner, 1989; Demeler et al., 2012), but differences in outcomes between sampling approaches remain poorly explored (Waller et al., 1981; Bryan and Kerr, 1988). The challenge is to develop a user-friendly sampling method that estimates pasture larval contamination with a precision that is acceptable in an epidemiological context. The aim of this study was (1) to compare two different sampling methods in terms of PLC and required time to sample herbage, (2) to assess the amount of variation in PLC at the level of sample plot, pasture and season, respectively and (3) to assess the adequate sample size for collecting herbage using random plots across pasture.

4.2 Materials and methods

4.2.1 Study design

In 2013, eight cattle pastures located in Flanders, Belgium were sampled in the morning during three consecutive seasons, spring (May/June), summer (August/September) and autumn (November/December). First season grazers grazed these pastures from April to November. The age of the animals at turn-out ranged from 6 to 24 months. At each sampling moment, pastures were sampled by two different methods, using both a double-crossed W-transect with samples taken every ten steps and four random located plots of 0.16 m^2 with collection of all herbage within the plot. The same protocol for L_3 recovery and L_3 identification was applied for all samples by a modified technique described by Taylor (1939) and expressed as number of L_3 per kg of dry herbage (L_3/kg DH). Climate data (precipitation (mm) and temperature (°C, minimum and maximum)) were registered daily by an automated weather station of the Royal Meteorological Institute, Belgium, located at maximum 34 km from the pastures (N 50°59’1.193”; E 3°48’43.548”).

4.2.2 Sampling methods

The first sampling method (method 1) was a modification of the technique described by Taylor (1939), in which researchers walked along two W - shaped transects across the pasture (Figure 4.1A). Every ten steps, four pinches of grass were collected in front of and behind the operator between index finger and thumb close to soil level. All pinches of grass were collected in plastic bags. The second sampling method (method 2) was based on sampling four random located plots of 0.16 m^2 using a wooden frame of 0.40 m by 0.40 m (Figure 4.1B). All herbage within the frame was collected and swards were cut as close as possible to soil level. The random location of the plots was determined using QGIS 1.8.0 software (QGIS Development Team 2012; http://qgis.osgeo.org). Grass samples of each plot were collected in separate plastic bags and analysed separately. For both methods, herbage within 1 meter of a faecal pat was not sampled. Bags containing the samples were transported in a cooling box to the lab.

The time required to sample by a single operator was registered during sampling for both methods. The time required to sample for method 1 was the time needed to collect an herbage sample through the double crossed-W pattern by one operator. The time required to sample for method 2 was the time required by one operator to sample four plots of 0.16 m^2 by cutting all the herbage within the plot, excluding the time needed to find the plot location.

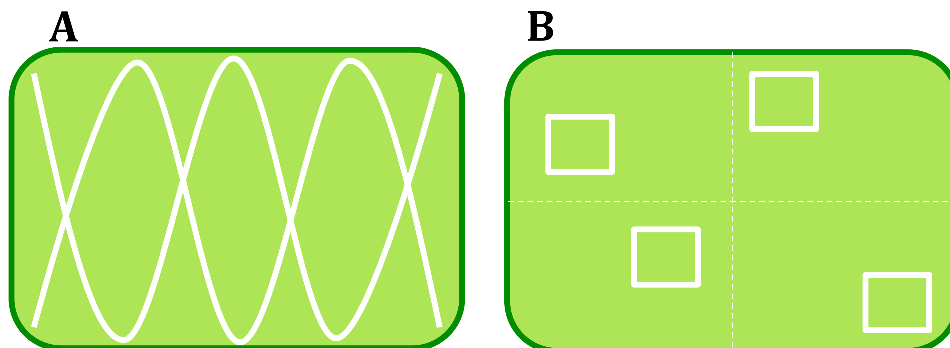

Figure 4.1. The different herbage sampling methods used in this study. **(A)** Method 1 consists of sampling along two W-shaped transects across pasture, **(B)** method 2 was based on sampling of four random located plots of 0.16 m^2 in each quadrant of the pasture.

4.2.3 Statistical analysis

Comparison of sampling method 1 and 2

To compare the results derived from the two sampling methods, all PLC were log-transformed (i.e. $\log_e$(PLC + 1)). For method 2, the average result of the 4 individual plot counts was used in this transformation. The effect of sampling method on PLC was analysed by a fixed effect model with as outcome variable the ratio of counts for method 1 over counts for method 2. Pasture and season were used as categorical fixed effects in this model. The time required to sample was compared between the two methods through a linear fixed effects model with sampling method, pasture surface area (ha) and the interaction between method and pasture surface area as fixed effects.

Estimating the variance components related to pasture, season and repeated measurements (Method 2)

A mixed model with pasture and season nested in pasture as random effects was fitted to estimate the variance components related to pasture, season and repeated measurements for the PLC obtained by sampling method 2.

Sample size requirement for sampling a pasture through random plots (Method 2)

Current formulae to calculate sample sizes are based on a normal distribution of the mean (central limit theorem), an approximation that may be very poor for skewed data such as L_3 counts. Therefore, we will use simulation to assess the required sample size *N* that allows assessing PLC with a predefined precision. In this simulation we assumed that L_3-counts obtained from a random plot *i* on a pasture *j* follow a negative binomial distribution parameterised by the mean pasture larval contamination μ_j and the level of aggregation k_j. From this negative binomial distribution a random sample of *N* plots was repetitively drawn (10,000 iterations). The mean L_3 count over *N* plots was determined for each iteation. As a measure of variation of the mean L_3 count, the width of the interval defined by the 2.5^{th}

and 97.5th percentile of the mean L_3 counts was used; 95% of the estimates will be contained on average in this interval. To gain more insights into the impact of N, μ_j and k_j on the precision of the estimate of PLC, we evaluated the precision for a wide range of values of N (4 to 40 plots), μ_j (11; 305; 2090 L_3/kg DH) and k_j (0.24; 1.41; 12.87). The values of μ_j and k_j were based on the observed mean, minimum and maximum of the mean and aggregation of L_3 counts obtained using method 2 described above.

4.3 Results

4.3.1 Climate data and pasture larval counts

Figure 4.2 shows the average PLC, the weekly air temperatures and the precipitation over the course of the survey. The average (± standard deviation (SD)) PLC found using sampling method 1 and 2 was 325 (± 479) and 305 (± 444) L_3/kg DH, respectively. Discrepancies between measurements of the two methods are often seen for samples collected at the same pasture and moment (Figure 4.3). The largest difference in PLC between methods was seen in spring, with 0 versus 969 L_3/kg DH measured by method 1 and 2, respectively. The most prevalent nematode species at the beginning of the pasture season was *Cooperia oncophora*, while towards the end *Ostertagia ostertagi* became more prevalent (Table 4.1).

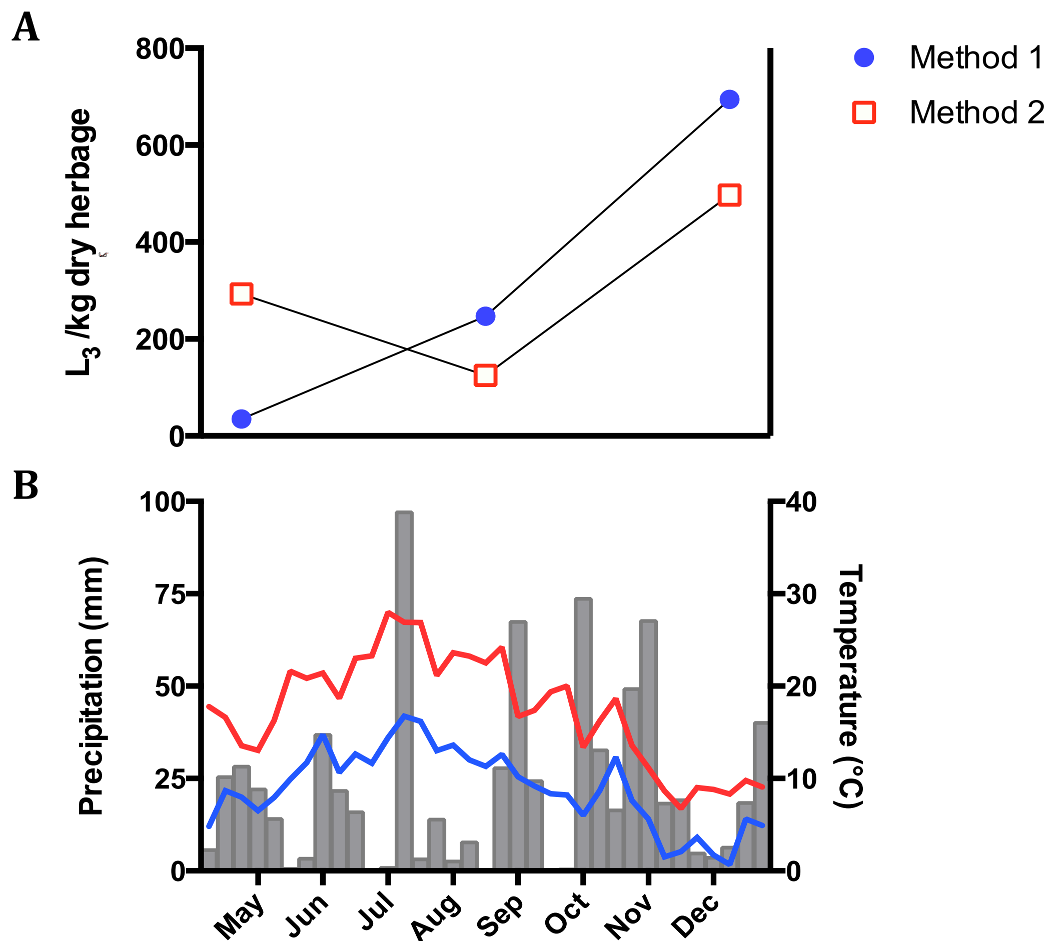

Figure 4.2 (A) Average pasture larval counts (L_3/kg dry herbage) obtained by two different sampling methods. Points and squares depict the results derived from samples collected using method 1 and 2, respectively. **(B)** Climate data registered from the 1st of May 2014 until 31st of December 2014 by an automated weather station of the Royal Meteorological Institute, Belgium (N 50°59'1.193"; E 3°48'43.548"). Bars represent the total weekly precipitation in mm, lines the weekly average minimum (blue line) and maximum (red line) temperatures in degree Celsius.

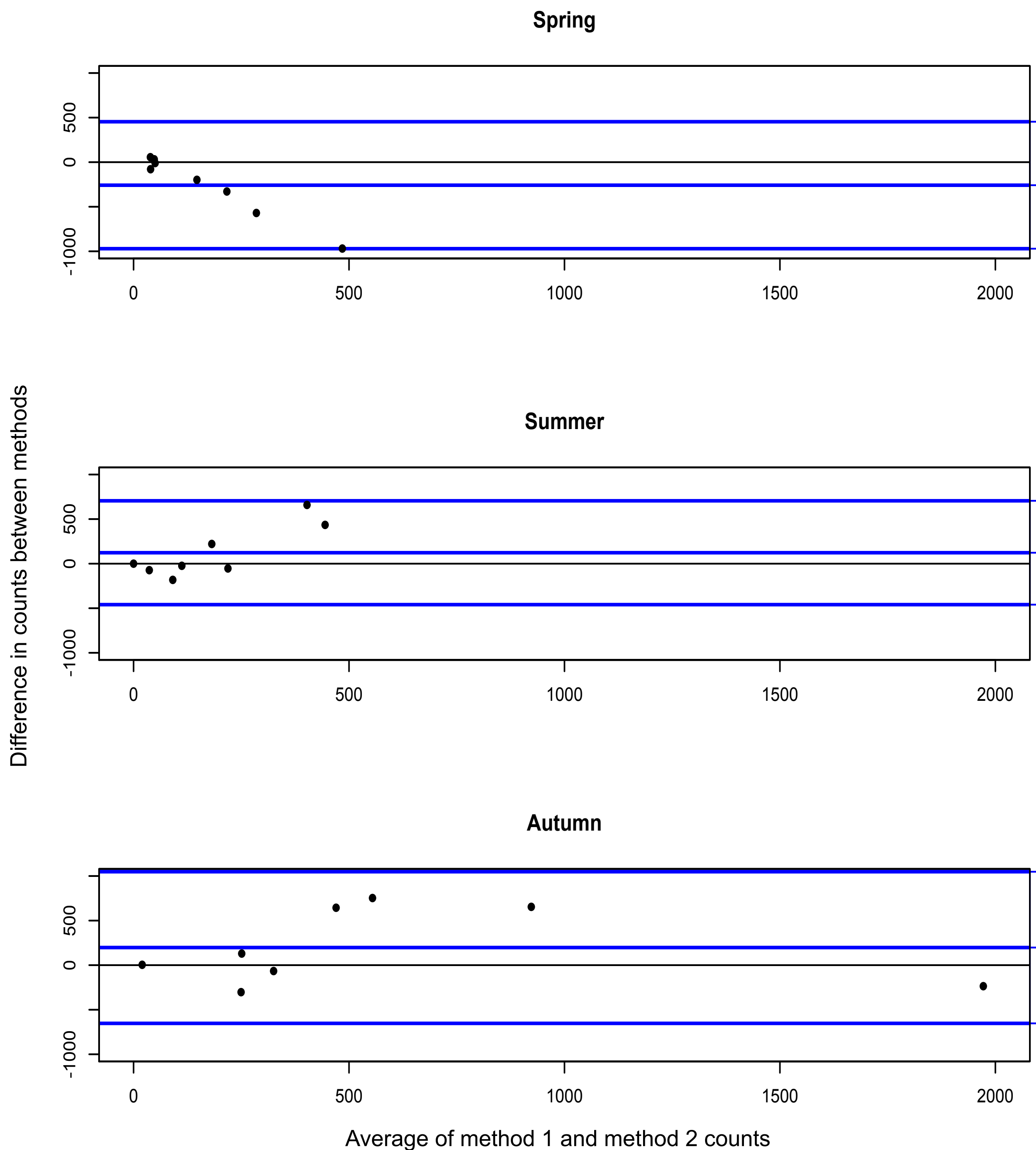

Figure 4.3 Difference plot for two sampling methods to measure pasture larval contamination (L_3/kg dry herbage) on 8 pastures at 3 different moments (spring, summer, autumn). Points depict the difference between the larval counts of the two sampling methods against the average of the methods' counts for each pasture. Horizontal lines are drawn at the mean difference and at the limits of agreement, which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences.

Table 4.1. Proportion of different nematode species found in the herbage samples during the pasture season.

	Spring (%)	**Summer** (%)	**Autumn** (%)
Ostertagia ostertagi	26	32	49
Cooperia oncophora	55	32	33
Nematodirus spp.	15	35	17
Oesophagostomum radiatum	3	0	0
Trichostrongylus axei	1	1	1

4.3.2 Comparison of sampling method 1 and 2

There was no significant difference (P = 0.38) in PLC between method 1 and method 2. The average ratio of log-transformed counts of method 1 over method 2 (95%CI) was 1.71 (0.53 – 5.54). For each method, the time required to sample by a single operator was registered during all samplings except for one. Collecting samples by method 1 took on average more time than using method 2 (26.0 ± 19.2 min versus 7.5 ± 1.8 min) and the time to sample increased with increasing pasture surface area for method 1 (P<0.001). For pastures with a surface area larger than 1 ha, the difference in sampling duration between methods was larger than for smaller meadows (Figure 4.4).

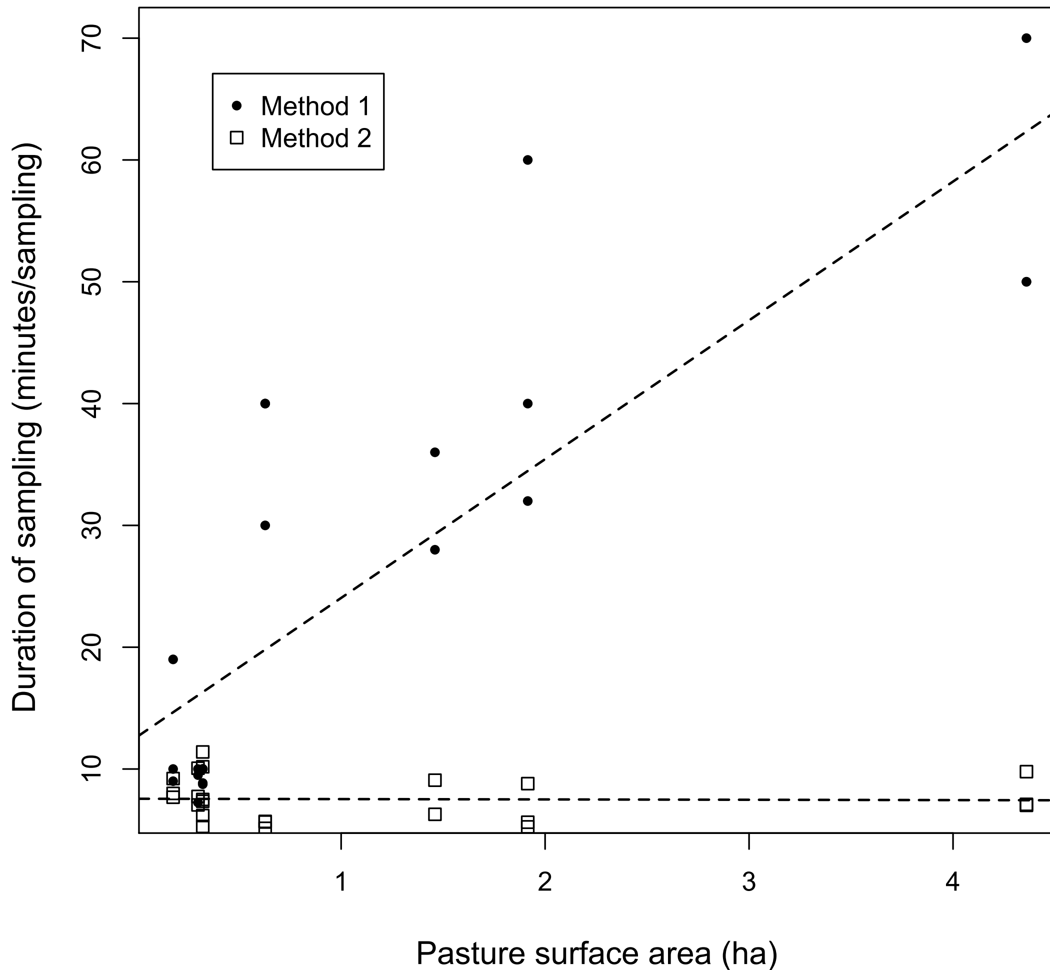

Figure 4.4 Time to sample (minutes) in relation to pasture surface area (ha) for two herbage sampling methods. Full circles depict data from samples collected along two W-shaped transects across pasture (method 1), squares depict data from sampling four random located plots on pasture (method 2).

4.3.3 Sources of variation in method 2: repeated measures, season and pasture

The variation in PLC from samples generated by random plot sampling was mainly due to the repeated measurements on the same pasture in the same season (residual variance component = 6.2), rather than due to pasture (variance component = 0.55) or season (variance component = 0.15).

4.3.4 Required sample size for sampling a pasture through random plots

The three curves in Figure 4.5A depict the sample size in relation to a measure for the variation of the estimated PLC (i.e. absolute width of the

interval defined by the 2.5th and 97.5th percentile of the mean L_3 count) for different levels of pasture larval contamination and different levels of aggregation. The variation of the estimated PLC was lower for pastures with a lower larval contamination and a lower aggregation level. However, in reality, pastures with a lower larval contamination are expected to have higher levels of aggregation and vice versa (based on Flota-Bañuelos et al., 2013). The most applicable scenarios in the field are considered here. Sampling 10 plots on a pasture with a mean contamination of 11 L_3/kg DH and a high level of aggregation (k = 0.24), resulted in a width of the CI of 27 L_3/kg DH. Sampling the same number of plots on a pasture with a mean pasture larval contamination of 305 and 2090 L_3/kg DH resulted respectively in a width of the CI of 315 and 720 L_3/kg DH when aggregation was respectively medium and low (k = 1.41; 12.87). To be able to compare the relative effect of pasture contamination and aggregation level on the acquired precision, Figure 4.5B shows the relation between sample size and the width of the interval defined by the 2.5th and 97.5th percentile relatively to the mean pasture larval contamination. Here, we see that for a pasture with a low contamination that is highly aggregated, more plots need to be sampled compared to a highly contaminated pasture with a low level of aggregation, to acquire the same relative precision.

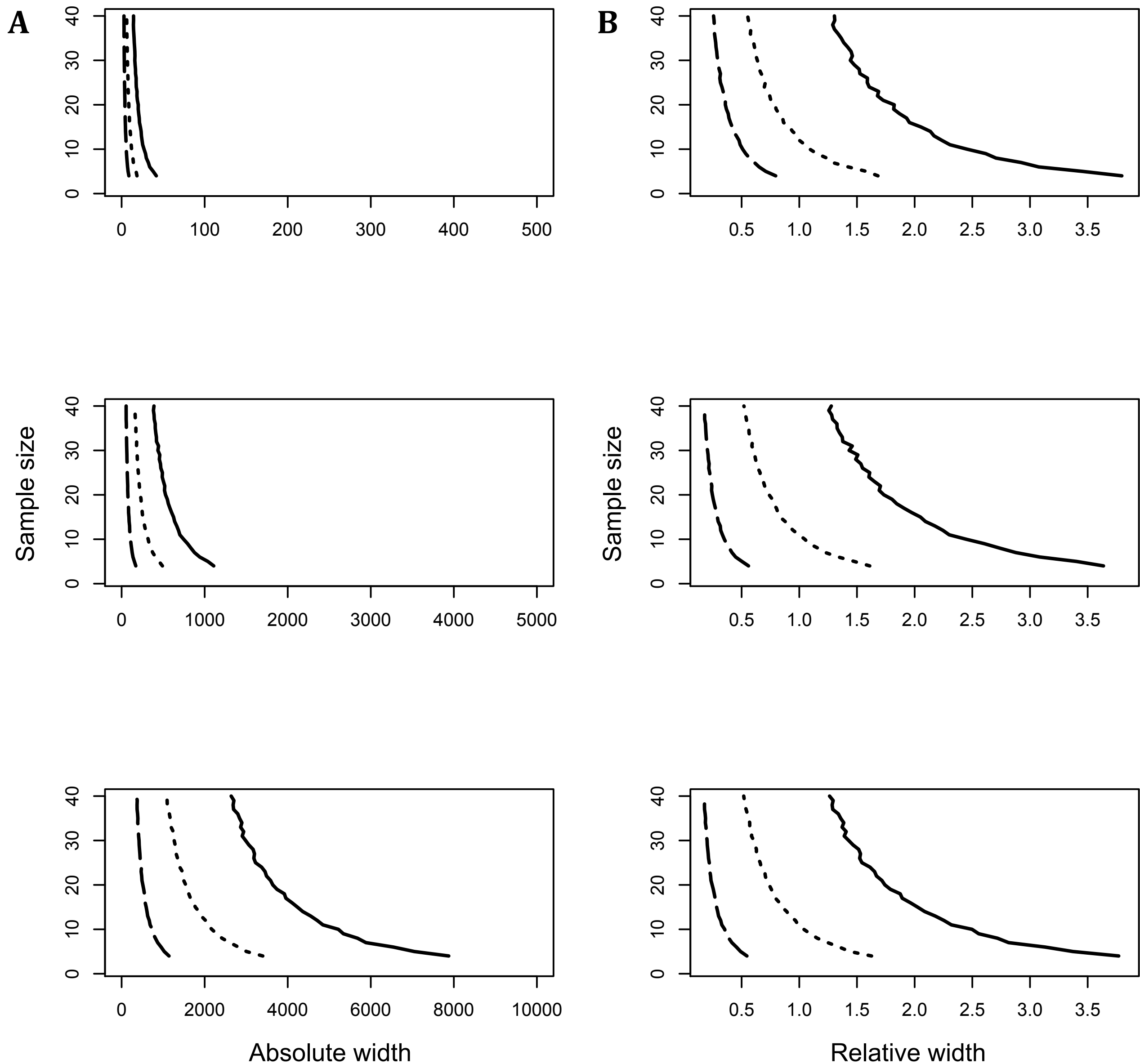

Figure 4.5 Results of a simulation assessing the required sample size (i.e. numbers of plots sampled per pasture) to obtain a predefined precision when measuring pasture larval counts. Sample size is given in relation to **(A)** the absolute width of the interval defined by the 2.5th and 97.5th percentile of the mean L_3 counts and **(B)** the relative width of the interval defined by the 2.5th and 97.5th percentile of the mean L_3 counts divided by the overall mean L_3 counts for different degrees of pasture larval contamination μ and different levels of aggregation k. Upper, middle and lower plots show the results for average pasture larval contaminations of respectively 11, 305 and 2090 L_3/kg dry herbage. Full, dotted and dashed lines depict the results for levels of aggregation of respectively 0.24, 1.41 and 12.87.

4.4 Discussion

Assessing the complex network of factors that determine the eventual pasture infection level and spatial distribution of L_3 is almost beyond resolution. Not only will host and parasite related parameters exert their influence (Crofton, 1954; Gruner and Sauve, 1982), but climate and weather events, such as heavy rainfall (Williams and Bilkovich, 1973; Bryan and Kerr, 1989), will also play an important role in larval dispersal. The use of PLC as routine diagnostic for pasture management will benefit from a standardised and simplified method to collect herbage samples. Regardless of efforts in improving and simplifying both the processing of herbage samples and the L_3 species identification (Demeler et al., 2012; Cassida et al., 2012; Sweeny et al., 2012; Bisset et al., 2014), the sampling approach has received very little attention to date.

No significant difference was found between PLC obtained by the two sampling methods. This suggests that method 2 could be further developed as a new herbage sampling method because it is far less time consuming. However, apparent discrepancies in PLC between methods were sometimes seen and the average ratio of method 1 counts over method 2 counts (1.71) could indicate that method 1 picks up more larvae. Two relevant technical differences that could underlie this observation are (1) that samples collected with method 1 were bigger in size and (2) that herbage was plucked and respectively cut for method 1 and 2. In contrast for method 2, the number of plucks taken and thus the amount of herbage collected, depends mostly on pasture area for method 1, however, no data is available on how magnitude of the herbage sample affects PLC results (Couvillion, 1993). Herbage samples collected by plucking contain more soil, which hinders L_3 recovery and identification (Crofton, 1954) and which can lead to unrepresentative larval counts, originating from the soil reservoir (Al Saqur et al., 1982; Callinan and Westcott, 1986; Demeler et al., 2012). Comparison of PLC obtained by tracer calves, the assumed golden standard, might be required to confirm a biological relevant difference between sampling methods.

Distribution patterns of trichostrongylid larvae on pasture are known to be aggregated (Crofton 1954; Donald, 1967; Gruner and Sauve, 1982; Boag et

al., 1989; Flota-Bañuelos et al., 2013). This is to be expected, as faecal pats are not evenly distributed across pasture (MacDiarmid and Watkin, 1972; Gruner and Sauve, 1982; Hirata et al., 2011; da Silva et al., 2013) and as most L_3 do not migrate considerable distances away from the faecal pat (Gruner and Sauve, 1982; Stromberg, 1997). The extent of the aggregation of faecal pats varies in time during the pasture season (MacDiarmid and Watkin, 1972; Gruner and Sauve, 1982) and so does the level of larval aggregation (Flota-Bañuelos et al., 2013). Aggregation of the distribution pattern of L_3 across pasture is especially important to take into account when reflecting on sample size. In our study, the levels of aggregation were relatively high as an average k of 1.41 was found. A recent study on a cattle pasture in Mexico reported lower levels of aggregation, with k values ranging from 1.8 to 167.2 (Flota-Bañuelos et al., 2013). Depending on the desired precision, the implementation of sampling procedures that are less labour intensive is hindered when L_3 are highly aggregated on pasture. However, it is expected that the aggregation level will decline as pasture larval contamination builds up, mitigating this effect for pastures with higher larval contamination levels. In the current study, a higher relative precision was acquired when estimating PLC on pastures with a high larval contamination and a low level of aggregation when the same sample size was applied. The use of PLC in validating nematode vaccination strategies and targeted control programmes and as input for nematode model predictions, however, requires good estimates for both low and high degrees of pasture contamination. Unfortunately, only limited data is available on the spatial distribution of trichostrongyle larvae on cattle pastures (Gruner and Sauve 1982; Flota-Bañuelos et al., 2013), as most studies consider data collected on sheep pastures (Crofton, 1954; Tallis and Donald, 1964; Donald, 1967; Boag et al., 1989). Extrapolation of results between host species is objectionable because differences in faecal morphology (i.e. volume, consistency, shape) and grazing behaviour are expected to influence the L_3 distribution. Therefore, more research on larval distribution patterns on cattle pastures under different geographical conditions is required to optimise herbage sampling.

To conclude, a big step in facilitating the widespread use of PLC would be to have a reliable sampling approach that can easily be performed by the

veterinarian or farmer. Sampling herbage from random plots across pasture seems a promising candidate for this, because both protocol complexity as well as time to sample will play an important role in succeeding. However, more insights are required into the aggregation of L_3 on pasture and the effect this could have on the required number of plots to sample by organising longitudinal samplings of cattle pastures on a high resolution scale.

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GENERAL DISCUSSION

The objective of this thesis was to develop a model framework for the parasitic phase of GIN infections in ruminants and to facilitate the collection of pasture larval count data, a crucial parameter for the implementation of this kind of models in the future. In this final chapter, the results and limitations of our work will be discussed along with opportunities for future research.

5.1 Parameterising the parasite component: key life history traits

Key life history traits of the parasitic phase of the GIN lifecycle were quantified by a systematic review and meta-analysis for two important nematode species found in cattle. Systematic review combined with meta-analysis is a powerful tool to obtain estimates for model parameters as it allows identifying and summarizing a large body of research evidence. The obtained parameter estimates are an important asset compared to former estimates for these life history traits, which are in general based on a limited number of experiments. Moreover, the uncertainty surrounding these estimates is clearly reported and the use of these estimates is not limited to the GLOWORM-PARA model, but can be important for other future modelling efforts in the field. Moreover, the protocol for the systematic review and meta-analysis can be a guideline to obtain parameter estimates for other nematode species, as it was aimed to report this protocol as transparent as possible. Despite the potential of the implemented protocol, there remain also a number of limitations. For example, it was not possible to obtain new estimates for all life history traits used in the GLOWORM-PARA model based on the available data (e.g. mortality of arrested larvae). Also, using the meta-analysis a precision weighted average was obtained for each key life history trait. Precision is an indication of the (un)certainty of the study observations, but is not necessarily a reflection of study quality. In the current meta-analysis, the influence of removing the study with the highest impact was checked for the datasets if applicable (unpublished analysis). Removing the highest impact study did not have a major effect on the overall estimate or on the relationship with moderator variables (results not shown), indicating that the analysis was robust in our case.

5.2 Parameterising the host component: dry matter intake and faecal production.

The two stages that connect the free-living and the parasitic phase of the GIN lifecycle are (1) the ingestion of infective larvae by the host and (2) the excretion of eggs in the environment. To model these, two host factors crucial for the connection need to be estimated, i.e. the dry matter intake and the faecal production respectively.

5.2.1 Dry matter intake

The infection rate for a grazing host is determined by the pasture larval contamination and the total amount of herbage ingested. Since pasture larval contamination is expressed as the number of L_3 per kg dry herbage, it is necessary to estimate the dry matter intake of grazing cattle to calculate the infection rate. A wide range of factors related to animal (e.g. body weight, age, parity, milk yield, pregnancy, disease), food (e.g. diet composition, digestibility, energy concentration, physical form), management (e.g. time of access to feed or pasture and frequency) or environmental characteristics (e.g. photoperiod and temperature) determine the dry matter intake of livestock (Ingvartsen, 1994). For growing cattle and dairy cows several methods are available to estimate the voluntary food intake ranging from simple regression models to more complex estimates incorporating a wide range of predictive factors (Ingvartsen, 1994; Vazquez and Smith, 2001). In GLOWORM-PARA, the dry matter intake was estimated using the relatively simple equations of MAFF (1975), which only takes bodyweight and, in case of adult cows, milk yield into account. Several considerations were made to decide on this approach. More complex equations that take more factors into account, might result in more precise estimates of the dry matter intake. The dry matter intake of grazing cattle is influenced by additional factors compared to the dry matter intake of confinement feeding systems (Vazquez and Smith, 2000). Factors such as pasture allowance (i.e. pre-grazing pasture mass x offered area), supplementation, herbage mass, sward height and herbage digestibility could be considered, since we focus on grazing hosts. The actual implementation of complex equations that include a wide variety of factors,

however, is limited by the detailed information needed to do so. In our case, the data needed to apply these more complex equations was not available. Moreover, the future use of a mechanistic model, such as GLOWORM-PARA, is not facilitated when detailed information of this extent would be needed as input. Further, a comparative study indicated that the simple equation of MAFF (1975) gives adequate and useful average predictions compared to more complex equations (Caird and Holmes, 1986).

For further development of the GLOWORM-PARA model, incorporation of more complex grazing behavioural aspects could be considered. An observed effect of GIN infections, for example, is the reduction of voluntary food intake (Kyriazakis et al., 1998), which could be taken into account (Vagenas et al., 2007). Also, adding an additional component that models grass growth in presence of grazing hosts could be considered, as the amount of available biomass will influence the GIN density and therefore infection pressure on pasture (Johnson and Parsons, 1985; Vaze et al., 2009). The question whether incorporating this additional complexity is an added value should be considered and can be underpinned by scenario analysis and sensitivity analysis.

5.2.2 Daily faecal production

The daily faecal production of cattle was needed to calculate the number of worm eggs excreted in the environment. Comparable to the estimate of the dry matter intake, an equation based on a feasible prediction factor, such as bodyweight, was preferred. The estimation of daily faecal production, however, is mainly assessed in the light of digestibility studies and not for the prediction of faecal production *per se* (e.g. Forbes et al., 1995; Ferret et al., 1999). Given the importance of manure management to plan storage facilities and comply with environmental regulations, other studies have focused on the prediction of manure excretion, which besides faeces also includes urine (ASAE, 2001; Nennich et al., 2005). No estimations of the daily faecal production based on body weight were found for cattle. Therefore, in GLOWORM-PARA, the daily manure production was first computed based on the equation of Nennich et al. (2005) from which the daily faecal production

was then obtained by correcting for the average proportion of urine found in cattle manure. This average proportion of urine was estimate based on data of 924 animals (Nennich et al., 2005; Weiss and St-Pierre, 2010; Orr et al., 2012; Massé et al., 2014). Future research needs to assess the daily faecal production based on easy-to-use predictors such as body weight. In experimental conditions the faecal production of grazing animals can be measured by collection of faeces from animals equipped with a harness and a faeces bag (Cordova et al., 1978).

5.3 Model validation using field observations: a tricky necessity.

As previously mentioned, models are simplified representations of real systems and, regardless of the level of complexity included, they will always remain simplified versions of reality. An important aspect of model development is therefore providing proof that the model is realistic enough to meet its intended purpose (Mayer and Butler, 1993). However, the lack of clear standards on which procedures and criteria need to be used to prove a model's validity is a serious obstacle (Rykiel, 1996). For parasitological models, previous authors have proposed the ability of a model to generate patterns that would be regarded as typical for a specific region by an experienced field worker as a criterion for validity (Smith and Grenfell, 1994; Smith, 2011). However, an objective assessment of such a criterion, seems to be difficult in practice and model behaviour may not be valid in other contexts e.g. for models intended to extrapolate current knowledge to alternative scenarios in less known contexts. Moreover, it needs to be shown that model output is a result of the right underlying mechanisms and not because it has been tweaked through calibration or smart parameterisation to give "typical patterns". Others have partially validated separate components of their model against results of artificial infection experiments (Dobson et al., 1990). This approach, however, cannot guarantee that all model components will interact adequately (Smith and Grenfell, 1994). Also, although artificial infection experiments can be very useful, they probably do not entirely reflect the situation of a natural infection. Therefore, one could conclude that when the intended aim of a model is to provide a tool that is capable of reproducing

epidemiological patterns of GIN infections observed in first season grazing cattle and that can be used to explore the impact of alternative climate and control scenarios on these patterns, the use of independent field observations for comparison to model output is a necessity to demonstrate a model's validity. The validation of the current model was therefore based on field observations.

Nevertheless, model validation by comparison with field observations is not straightforward. The needed data is quite specific, has a high level of detail and requires intensive data collection. As a result, these kinds of data are often not readily available (Smith and Grenfell, 1994). For the validation of GLOWORM-PARA, longitudinal parasitological data was collected on different commercial farms during different years. Besides the problem of data availability, however, other limitations and methodological issues need to be considered. As a start, the data originates from a rather limited area, the region of Flanders (Belgium) and the data collection was performed on a monthly or two-monthly basis throughout the grazing season, restricting the number of observations that can be used for comparison with model output in the regression analysis. Further, the aim of collecting such an extended set of field observations was to create data that embodies a wide range of farm situations and varying infection levels. Nonetheless, another obstacle in the use of field observations is the dependency on multiple factors that determine the actual infection levels in a herd. Mainly because of weather conditions, the observed FEC in 2013 were in general low and the observations did not result in data with a wide range of varying infection levels. Therefore, additional data from previous field trials performed at the Laboratory for Parasitology (Ghent University) was used for the validation of grazing behaviour.

The fact that model output is in general sensitive to initial model input (i.e. pasture contamination level, date of turn-out, host age at turn-out, immune status of the animals) needs also to be taken into account. For GLOWORM-PARA, daily values on the pasture contamination level were needed as model input. To obtain these for the entire simulation period, polynomial interpolation was performed on the discrete sets of monthly observations, which might not entirely be in correspondence with real pasture infection levels and could therefore potentially induce bias. Lastly, it would be interesting if model output

on several variables could be validated. In the case of GLOWORM-PARA, however, FEC was the only variable of interest because this is the only variable that can be measured repeatedly. Other variables, such as the number of adult parasites, can only be measured once at necropsy.

Taking these considerations into account, it should be noted that model validation is a tool and not an objective on its own. It probably is indeed, as Smith and Grenfell (1994) stated, unreasonable to expect precise correspondence between a single set of observations and model output. Additionally, measuring error as a source of uncertainty in field observations makes perfect consistency between predictions and observations even more difficult. The recovery of L_3, for example, depends on different variables such as climate and laboratory technique used (Demeler et al., 2012) and considerable variation is seen between repeated measurements (Boag et al., 1989; Couvillion, 1993). Likewise, the measurement of FEC is subjected to considerable variation (Gasbarre et al., 1996; Levecke et al., 2011; Morgan, E. R. unpublished data). The question of how much deviation between observations and model output is acceptable and of when a model is in fact successfully validated, remains difficult to answer. The implementation of a combination of validation methods, such as visual and statistical validation, needs at least to be aimed for (Mayer and Butler, 1993; Rykiel, 1996).

5.4 Continuing the model development cycle for GLOWORM-PARA: future prospects.

GLOWORM-PARA was successful in simulating infection patterns of first season grazers for *O. ostertagi* and *C. oncophora*, but the framework is flexible and allows future adjustments to further improve model performance. The model framework encompasses a separate entity for immunity, which provides the opportunity to incorporate additional complexity in the acquisition of immunity at nematode species level when needed or as our understanding of the mechanism underlying immunity increases. For *C. oncophora*, for example, future inclusion of density dependence, worm expulsion or age-related immunity may be beneficial. Also, if suitable data become available, fine-tuning the specific influence of immunity on the separate life history traits

might be interesting as the underlying mechanisms of these effects probably differ (Claerebout and Vercruysse, 2000).

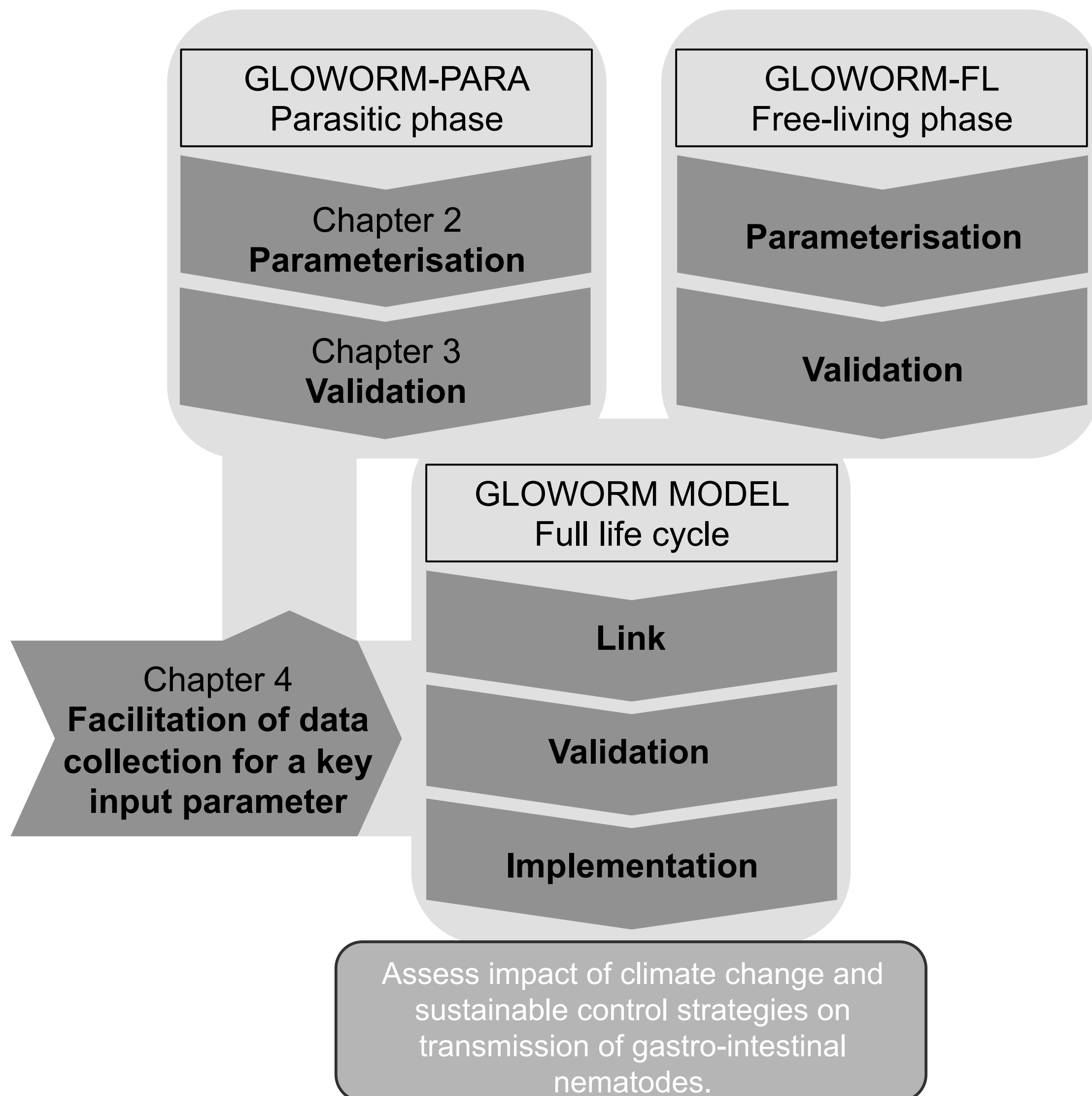

Figure 5.1 Overview of how the achieved objectives of this thesis are linked to future research plans and the overall research goal.

GLOWORM-PARA simulates the parasitic phase of the GIN lifecycle, but in order to explore the impact of different control and management approaches on parasite epidemiology, a model that embodies the full lifecycle is needed. GLOWORM-PARA was therefore developed to complement a recently developed and validated model of the free-living phase, i.e.

GLOWORM-FL (Rose et al., 2015). Future work is required to link these two models and, despite that these two models were both validated, further validation of the full life cycle model is needed to asses whether the two model components interact adequately (Figure 5.1). Taking the previous considerations into account, it is recommended to collect new field observation data for this. Different factors drive the different life cycle phases and while immunity is a key driver of the parasitic phase, meteorological factors are the main drivers of the free-living phase. Therefore, data on temperature and precipitation will be used as additional input for the full lifecycle model. Simulations of the full life cycle model start with the ingestion of infective larvae by the host. These infective larvae develop into male and female adults that reproduce. Eggs are deposited on pasture and develop in to infective larvae within the faecal pat. Finally, the infective larvae move from the faeces to the pasture where they can migrate into the soil or on to the herbage from where they can be picked up by a host. In contrast to the simulations ran for the parasitic phase alone (GLOWORM-PARA), it is not necessary to interpolate the pasture larval contamination data, since only the initial pasture contamination needs to be known. For the validation, pasture larval contamination could therefore be used as an additional variable of interest, alongside FEC.

The resulting full life cycle model can be used to compare the impact of different control strategies and farm management situations on parasite epidemiology under varying climate conditions. It might also be worth including an additional model component that simulates grass growth to underpin the incorporation of these management aspects. Ideally, it would be very useful to explicitly incorporate the changes in management as a result of climate change, as Morgan (2013) proposed, to assess the impact of mitigation and adaptation strategies. However, an adequate knowledge on what drives farmer’s behaviour is currently lacking to do this. Advice on worm control currently focusses on targeted treatments of herds and individual animals. Part-treatment of a herd, e.g. leaving 10% untreated, can also be incorporated into mean-field models such as the GLOWORM-PARA model. Modifying the model to an individual-based model that incorporates demographic stochasticity, is also a possibility for future improvement, but the

main challenge faced to do so is to calibrate such models with realistic and meaningful parameter values and distributions.

5.5 On-farm predictions: a dream or the future?

Since several years, a strict, rather theoretical, distinction between 'illustrative models' and 'forecasting models' is made in reviews of parasitic transmission models (Smith and Grenfell, 1994; Cornell, 2005; Smith, 2011). These authors question the potential and utility of the 'forecasting models', which ideally are able to predict the events on any specific farm in a specific year (Cornell, 2005; Smith, 2011). Indeed, when looking at the progress made over the years, existing models are, until now, mainly used for illustrative purposes rather than predictive purposes. However, it is not a delusion to believe that predictions of transmission models can provide farm-level decision support for worm control in the future. Parasite dynamics are driven by multiple factors and the complexity of pinning down key drivers and the various sources of uncertainty make precise predictions difficult (Cornell, 2005). However, reliable predictions can be achieved within the range of uncertainty for measurements like FEC, as is shown by the GLOWORM-PARA model. Moreover, validation based on field observations is in fact site-specific prediction, with that difference that it is retrospective.

The required input data to drive models that forecast infection patterns at farm-level needs to be farm specific. In the past, the problem of data availability has often been raised as an important limitation to site-specific forecasting. The trend of performing on-farm measurements and diagnostics, however, provides important possibilities for the future. More and more farmers, for example, have on-farm weather stations. This is currently the case for the U.K. (Rose H., personal communication), but it can be expected that this trend will slowly make its way to other countries (e.g. www.forwardfarming.com). Currently, innovative tools for on-farm assessment of parasite infections are being developed, which allows farmers and/or veterinarians to perform FEC *in situ* (e.g. http://fecpakg2.com). Such tools can strongly facilitate the verification of decision-support and model predictions. As pasture infectivity at the start of the grazing season remains an important

input parameter, efforts to provide practical estimates based on grazing history and climate need to be encouraged as this will underpin the future implementation of models.

To facilitate site-specific forecasting, attempts must also be directed at improving the utility and implementation of such models by reaching out to other researchers and stakeholders such as veterinarians and farmers. So far, most achievements concerning transmissison models of GIN infections have not reached further than a peer-reviewed scientific publication resulting in a loss of know-how for these models afterwards. When looking a bit broader, however, examples of inspiring attempts to bridge the gap between researchers and stakeholders exist. Most examples do not involve transmission models, but are websites for veterinarians and farmers that provide information and advice on worm control which sometimes is underpinned by decision trees (e.g. www.scops.org.uk, www.parasit'info.com, www.parasietenwijzer.nl, www.wormboss.com.au, www.paracalc.be). As they probably are the most successful examples of knowledge transfer in parasite epidemiology until now (Morgan, 2013), they form a basis and inspiration for future decision support. In the long run, incorporation of transmission models, or at least some kind of decision support for worm control, in farm management software should be looked into to reach more stakeholders. To underpin this knowledge transfer, the identification and assessment of user-needs is crucial. For example, the Laboratory for Parasitology organised two focus group meetings with Flemish veterinarians and cattle farmers in 2014, to explore the user-needs for software applications on worm control. These meetings showed that the available tools only partially address the user-needs and that both farmer and veterinarian would prefer to have several tools grouped into one general application.

5.6 Concluding remarks

This thesis provides estimates for key life history traits of two important cattle nematodes, presents a new framework to simulate the parasitic phase of the GIN life cycle in ruminants and explores the potential of facilitating the assessment of pasture larval contamination, an important input parameter of

nematode transmission models. This thesis addressed several needs in the field of modelling parasite dynamics and will therefore contribute to their future development. Transmission models can provide improved insights of parasite epidemiology under altered conditions and support evaluation of alternative control strategies. In the future, the implementation of transmission models as site-specific decision support tools for the control of GIN seems promising.

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SUMMARY

Gastro-intestinal nematodes (GIN) are one of the great threats for farmed ruminants worldwide. These parasites are highly prevalent and infections with GINs are an important limitation to animal performance. Moreover, reports on climate change and anthelmintic resistance raise the question whether the current control methods will remain sustainable in the future. Mathematical models that simulate the dynamics of GIN infections have great potential to provide improved understanding of parasite epidemiology under altered conditions and to underpin the development of alternative parasite control strategies.

In **chapter 1**, first the general epidemiology of GIN in ruminants is discussed to provide insight in the dynamics and underlying drivers of the host-parasite interaction. Host immunity, weather and farm management are shown to be significant drivers of parasite epidemiology. The second part of chapter 1 discusses the evolution of both hosts and parasites during the past half century, the expected trends to come and the underlying drivers of these anticipated changes. A major challenge for the cattle industry in the future will be to ensure food availability and safety in a way that is ethically and environmentally acceptable, which will need to happen against a background of increased climate variability. To maintain or even increase future production levels, the control of GINs will remain important, but is challenged by the need to decrease the use of anthelmintic products and by climate change affecting current parasite epidemiology and farm management. However, the existence of interventions that mitigate climate change and increase productivity at the same time, provide important opportunities.

In the second part of this chapter, the value of transmission models to improve our understanding of parasite epidemiology under changing conditions and to facilitate the development of control strategies is discussed. Key terms encountered in the field of parasitic disease modelling are explained and the development process of these models is given. An overview of the available models for GIN infections in ruminants provides insights into the needs for this field of research. A large amount of research is devoted to the subject of modelling GIN in farmed ruminants and several applications of these models exist (analysis, simulation, education). The majority of the models have considered various nematode species of sheep,

while fewer models exist for cattle and have focused on only one nematode species (i.e. *Ostertagia ostertagi*). The development of models for GINs in general is often hindered by the lack of suitable data for parameterisation. Further, no standardised approach exists for the validation of transmission models and validation based on sufficient field observations is limited.

The overall objective of this PhD project was to develop a generic framework for a mechanistic transmission model that simulates the parasitic phase of the GIN lifecycle in farmed ruminants. Further, facilitation of the collection of pasture larval count data, a key input parameter, was explored.

Chapter 2 quantifies the main life history traits of the parasitic phase for *O. ostertagi* and *Cooperia oncophora* through systematic review and meta-analysis and assesses the potential influences associated with the effect of immunity on these traits. The main parameters determining parasite density during the parasitic phase are the larval establishment rate or pre-adult mortality, the hypobiosis rate, adult mortality and female fecundity. 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 conducted at the Laboratory for Parasitology of Ghent University, Belgium. Overall inverse variance weighted estimates were computed for each of the traits through random effects models. To our knowledge, this systematic review is the first to summarize the available data on the main life history traits of the parasitic phase of *O. ostertagi* and *C. oncophora* and provides novel estimates for the parameterization of life cycle-based transmission models.

Chapter 3 presents a flexible model framework (GLOWORM-PARA) developed for the parasitic phase of GINs infecting ruminants. The framework can be applied to a range of GIN species and is parameterised and thoroughly validated for first season grazing calves infected by two species that are of major importance in cattle, i.e. *O. ostertagi* and *C. oncophora*. To our knowledge, no previous attempt has been made to model *C. oncophora*. For *O. ostertagi,* GLOWORM-PARA incorporates important improvements to the existing models such as data-driven parameterisation of the rate of acquisition of immunity based on cumulative exposure and the incorporation

of host grazing behaviour. Both the parameterisation and validation of these models were backed by extensive datasets obtained from various sources and acquired over decades of parasitological research. This represents the most comprehensive and thorough validation of GIN models to date. The model was able to generate the general patterns of faecal egg counts seen in first season grazing cattle throughout the grazing season. The estimation of the immune response rate from field observations was preferred over fitting the immune response rate to get meaningful predictions of acquired immunity. Linear regression of predictions against observations showed that incorporating host grazing behaviour resulted in an important improvement of model performance and is therefore likely to be important in the transmission of GIN.

Assessing levels of pasture larval contamination is frequently used to study the population dynamics of the free-living stages of parasitic nematodes of livestock and the abundance of infective larvae (L_3) on pasture is an important input parameter for GLOWORM-PARA. Direct quantification of L_3 on herbage is the most applied method to measure pasture larval contamination, but herbage collection remains labour intensive. **Chapter 4** compares two different sampling methods in terms of pasture larval count results and time required to sample, to assess the amount of variation in larval counts at the level of sample plot, pasture and season, respectively and to calculate the required sample size to assess pasture larval contamination with a predefined precision using random plots across pasture. Eight young stock pastures of different commercial dairy herds were sampled in 3 consecutive seasons during the grazing season (spring, summer and autumn). On each pasture, herbage samples were collected through both a double-crossed W-transect with samples taken every ten steps (method 1) and four random located plots of 0.16 m^2 with collection of all herbage within the plot (method 2). The average (± standard deviation (SD)) pasture larval contamination using sampling method 1 and 2 was 325 (± 479) and 305 (± 444) L_3/kg dry herbage (DH), respectively. Large discrepancies in pasture larval counts of the same pasture and season were often seen between methods, but no significant difference (P = 0.38) in larval counts between methods was found. Less time was required to collect samples with method 2. This difference in

collection time between methods was most pronounced for pastures with a surface area larger than 1 ha. In the future, herbage sampling through random plots across pasture (method 2) seems a promising method to develop further as no significant difference in counts between the methods was found and this method was less time consuming. However, more insights are required into the aggregation of L_3 on pasture and the effect this could have on the required number of plots to sample.

Chapter 5 discusses the results and limitations of this work along with opportunities for future research. The integration of GLOWORM-PARA with a complementary model which simulates the free-living stages of GINs, GLOWORM-FL, should lead to a full life cycle based model in further research. To improve the link between the free-living and the parasitic phase, future research needs to assess the daily faecal production based on easy-to-use predictors such as body weight. The incorporation of a component that models grass growth can provide the needed complexity to account for different farm management situations and to underpin meaningful larval infection rates. Several questions remain concerning the implementation of transmission models as site-specific decision support tools for nematode control. A proposed approach to achieve better and more applied modelling is to gradually refine generic models with the needed amount of biological detail. Obtaining relevant and realistic parameter estimates and integrating these in generic models might be a good step to achieve the right balance between generality and specificity. Efforts to facilitate data quality and collection should be encouraged, as this is fundamental to make progress and underpins the future implementation of models. Future research should also focus on how to improve knowledge transfer to the end-users and to identify user-needs.

SAMENVATTING

Infecties met maagdarmwormen zijn wereldwijd een belangrijke economische verliespost voor de rundveesector. Een verminderde gewichtsaanzet en een gedaalde melkproductie zijn de belangrijkste uitingen van deze productieverliezen. De toenemende problematiek van anthelminthicumresistentie en klimaatverandering doen echter de vraag rijzen of de huidige wormcontrole duurzaam is naar de toekomst toe. Mathematisch transmissiemodellen die de dynamiek van deze parasitaire infecties weerspiegelen, kunnen zorgen voor een beter begrip van de parasitaire epidemiologie onder veranderde omstandigheden en kunnen helpen om de beste controleaanpak te identificeren en te evalueren.

In **hoofdstuk 1** werd de algemene epidemiologie van maagdarmwormen in herkauwers besproken om een beeld te geven van de dynamiek en de onderliggende drijfveren van de gastheer-parasiet interactie. Klimaat en bedrijfsmanagement hebben beide een grote invloed op de epidemiologie van deze parasieten. Het tweede gedeelte van hoofdstuk 1 beschrijft de evolutie die zowel gastheer als parasiet hebben ondergaan gedurende de voorbije 50 jaar en bespreekt de verwachte veranderingen die zullen volgen en de hiervoor onderliggende oorzaken. De productie van voldoende veilig voedsel op een manier die ethisch en ecologisch verantwoord is in een kader van verhoogde klimaatvariabiliteit, zal de grootste uitdaging zijn voor de rundveesector in de toekomst. De controle van maagdarmwormen blijft de komende jaren belangrijk om de huidige productieniveaus te garanderen en eventueel te verhogen. De noodzaak om het anthelminthicumgebruik terug te schroeven en de potentiële impact van klimaatsveranderingen op de huidige parasitaire epidemiologie kunnen er echter voor zorgen dat de bestrijding van maagdarmwormen een uitdaging wordt in de toekomst. Het bestaan van maatregelen die zowel het effect van klimaatsverandering tegengaan als de productiviteit verhogen, bieden belangrijk opportuniteiten voor de sector. Tot slot worden op het einde van hoofdstuk 1 transmissiemodellen besproken als hulp om ons begrip van de parasitaire epidemiologie onder wijzigende omstandigheden te verbeteren en om de ontwikkeling van innovatieve controlestrategieën te ondersteunen. Zowel de gebruikte sleuteltermen uit het vakgebied als het ontwikkelingsproces van dergelijke parasitaire transmissiemodellen worden verhelderd. Verder wordt ook een overzicht

gegeven van de transmissiemodellen die beschikbaar zijn om maagdarmworminfecties van herkauwers te simuleren, met het doel inzichten te gegeven in de bestaande noden van dit onderzoeksgebied. Er is reeds veel onderzoek gebeurd rond het modelleren van maagdarmwormen en er bestaan verschillende toepassingen van deze modellen (analyse, simulatie, educatie). Het merendeel van de modellen handelt over nematodesoorten in schapen, terwijl slechts een klein aantal modellen bestaat voor rundvee. Bovendien ligt bij deze rundveemodellen de focus op slechts één nematodenspecies, nl. *O. ostertagi*. De ontwikkeling van modellen voor maagdarmwormen is in het algemeen gelimiteerd door een gebrek aan geschikte data voor parameterisatie. Bovendien bestaat er geen gestandaardiseerde aanpak voor de validatie van deze transmissiemodellen en zijn de bestaande modellen gevalideerd op basis van onvoldoende veldobservaties.

De algemene doelstelling van dit doctoraatsonderzoek was een mathematisch transmissiemodel te ontwikkelen dat de parasitaire fase van de levenscyclus van maagdarmwormen in herkauwers simuleert. Verder werd nagegaan of de methode om de larvaire weidebesmetting te meten, kan worden vereenvoudigd aangezien dit een belangrijke input parameter is.

In **hoofdstuk 2** worden de sleutelfactoren van de parasitaire fase gekwantificeerd voor *O. ostertagi* en *C. oncophora* aan de hand van systematische literatuur review en meta-analyse. Bovendien werd nagegaan welke variabelen een effect hebben op deze sleutelfactoren. De vier sleutelfactoren die de parasitaire fase karakteriseren zijn de larvaire ontwikkeling (het aantal opgenomen infectieve larven dat het volwassen stadium bereikt), de hypobiose (het aantal opgenomen infectieve larven dat in hypobiose gaat), de mortaliteit (het aantal volwassen wormen dat afsterft gedurende de infectie) en de fecunditeit (het aantal eitjes dat een volwassen vrouwtje legt). Er werd een systematische review uitgevoerd die studies omvatte van 1962 tot 2007. In deze studies werden helminth-naïeve dieren artificieel geïnfecteerd met *O. ostertagi* en/of *C. oncophora*. De dataset werd verder uitgebreid met data van ongepubliceerde studies die werden uitgevoerd in het Laboratorium voor Parasitologie van de Universiteit Gent. Op basis van een random effecten model werd voor iedere sleutelfactor een

gewogen gemiddelde waarde berekend. Dit is de eerste systematische review die alle beschikbare data verzameld en samenvat voor sleutelfactoren van de parasitaire fase van *O. ostertagi* en *C. Oncophora*. Deze studie geeft nuttige parameterschattingen voor de parameterisatie van mechanistische transmissiemodellen voor maagdarmwormen.

Hoofdstuk 3 beschouwd de ontwikkeling van een transmissiemodel (GLOWORM-PARA) voor de parasitaire fase van maagdarmwormen dat toegepast kan worden voor verschillende nematodensoorten in herkauwers. Het model werd in deze thesis geparameteriseerd en gevalideerd voor twee belangrijke nematodensoorten van eerste weideseizoenskalveren, i.e. *O. ostertagi* en *C. oncophora*. Dit is het eerste model dat beschikbaar is voor *C. Oncophora* en voor *O. ostertagi* bevat GLOWORM-PARA belangrijke verbeteringen ten opzichte van de bestaande modellen. De parameterisatie van de immuniteitsopbouw in het model is gebeurd aan de hand van relevante data op basis van een cumulatieve blootstelling aan L_3 en het model houdt bovendien rekening met het graasgedrag van de gastheer. De parameterisatie en validatie van GLOWORM-PARA gebeurde aan de hand van een uitgebreide datasets afkomstig van verschillende bronnen en is gebaseerd op verschillende decennia van parasitologisch onderzoek. Dit is, voor zover wij weten, de meest uitgebreide en grondige validatie van een transmissiemodel voor maagdarmwormen tot op heden. In het algemeen was het model in staat de patronen van fecale ei-uitscheiding door eerste weideseizoensdieren te reproduceren. De modelversie waarbij de immuunrespons werd geschat op basis van veldwaarnemingen wordt verkozen boven de modelversie waarbij de immuunrespons werd gefit aan velddata. Lineaire regressie waarbij predicties en observaties worden vergeleken toonde aan dat de integratie van graasgedrag van de gastheer resulteert in een belangrijke verbetering van de modelprestaties.

Het meten van de larvaire weidebesmetting is belangrijk om de populatiedynamiek van de vrij-levende nematodenstadia te bestuderen. Verder is het een belangrijke inputparameter voor mechanistische transmissiemodellen zoals GLOWORM-PARA. Directe kwantificering van de infectieve L_3 op het gras is de meest toegepaste methode om de larvaire weidebesmetting te meten. De staalcollectie op de weide blijft echter een

arbeidsintensieve aangelegenheid. Het doel van **hoofdstuk 4** was daarom om twee verschillende bemonsteringsmethoden te vergelijken in termen van resultaat en de tijd die nodig is voor de collectie van de stalen. Bijkomend werd nagegaan hoeveel van de variatie in de resultaten kan worden toegekend aan staalnameplaats, weiland en seizoen, respectievelijk, en werd de vereiste steekproefgrootte berekend met een vooraf gedefinieerde precisie voor de meting van de weidebesmetting aan de hand van willekeurige gelokaliseerde staalnameplaatsen. Hiervoor werden 8 jongveeweiden van verschillende commerciële melkveebedrijven bemonsterd in 3 opeenvolgende seizoenen (voorjaar, zomer en herfst) gedurende één weideseizoen. Op elk weiland, werden grasstalen verzameld door middel van twee verschillende bemonsteringsmethoden. Methode 1 bestond uit het volgen van een dubbel gekruist W-patroon waarbij om de tien stappen stalen werden genomen. Methode 2 bestond uit het bemonsteren van vier willekeurige gelokaliseerde staalnameplaatsen die elk 0.16 m^2 groot waren, waarbij al het gras binnen de staalnameplaats werd verzameld. De gemiddelde larvaire weidebesmetting (± SD) gemeten aan de hand van bemonsteringsmethode 1 en 2 bedroeg respectievelijk 325 (± 479) en 305 (± 444) L_3/kg droge stof. Ondanks dat grote verschillen die werden gemeten in larvaire besmetting op dezelfde weide in hetzelfde seizoen aan de hand van de verschillende bemonsteringsmethoden, kon geen significant verschil (P = 0.38) tussen de methoden worden aangetoond. De staalname nam minder tijd in beslag wanneer deze werd uitgevoerd aan de hand van methode 2 en dit verschil was het meest uitgesproken voor weilanden die groter waren dan 1 ha. Grascollectie aan de hand van willekeurige gelokaliseerde staalnameplaatsen (methode 2) lijkt een veelbelovende methode om verder te ontwikkelen in toekomstig onderzoek, aangezien geen significant verschil met de originele methode werd gevonden en methode 2 minder tijdrovend is.

In **hoofdstuk 5** worden de resultaten en de beperkingen van dit werk besproken samen met de mogelijkheden voor toekomstig onderzoek. De integratie van GLOWORM-PARA met een complementair model dat de vrij-levende fase simuleert (i.e. GLOWORM-FL) moet in verder onderzoek leiden tot een model dat de volledige levenscyclus van maagdarmwormen omarmt. Verder onderzoek naar de dagelijkse fecale productie op basis van eenvoudig

te gebruiken parameters, zoals lichaamsgewicht, kan zorgen voor een goede verbinding tussen de parasitaire en vrij-levende fase. Bijkomende incorporatie van een component die de grasgroei modelleert kan ervoor zorgen dat verschillende managementsituaties nog beter in rekening gebracht worden en dat het inbouwen van zinvolle larvaire infectieniveaus ondersteund wordt. Transmissiemodellen bieden een meerwaarde om onze inzichten in de parasitaire epidemiologie onder alternatieve omstandigheden te verbeteren en om de ontwikkeling van innovatieve bestrijdingsstrategieën te ondersteunen. Het gebruik van transmissiemodellen als ondersteunende hulpmiddelen voor bedrijfsspecifieke wormcontrole in de (nabije) toekomst lijkt veelbelovend. Het feit dat meer en meer metingen en diagnostiek gebeuren op de boerderij zelf, levert belangrijke mogelijkheden om bedrijfsspecifieke inputdata te genereren. Verder moet toekomstig onderzoek zich richten op het verbeteren van kennisoverdracht naar de eindgebruikers en het identificeren van de gebruikersbehoeften.

APPENDIX

Appendix A: Estimation of prepatent periods

Ostertagia ostertagi

The prepatent period is defined here as the time to the mean of the day that eggs first appear in the faeces and the day that egg counts are maximal after a single infection. A total of 7, 18-25 week old male calves with no previous experience of infection were housed in parasite-free conditions and infected with 30,000 L_3 (day 0). The infections took place over a period of 3 years for reasons other than the collection of data for parameter estimation. Faeces was collected at intervals from day 15 using a harness and nematode eggs were counted using the modified McMasters method. Occasionally egg counts were carried out twice daily but only the afternoon egg count is used here. Where peak counts were tied, the first count was used as the timing of peak egg output. If eggs were recovered on day 15, the timing of first appearance of eggs was assumed to be day 14. The results of the egg counts can be found in Table 6.1.

Cooperia oncophora

Details of infections are identical to *O. ostertagi* except that data were available for a total of 4 calves. Furthermore, egg counts were high for calves 1 and 2 on day 15 and therefore the timing of first appearance of eggs in faeces was assumed to be day 13. The results of the egg counts can be found in Table 6.2.

Table 6.1 Egg counts for naïve calves infected with 30,000 *O. ostertagi* L_3. The timing of peak egg output used to estimate the prepatent period is denoted with an asterisk (*).

	Animal 1 April 2012	Animal 3 May/June 2012	Animal 4 May/June 2012	Animal 5 Oct/Nov 2012	Animal 6 Oct/Nov 2012	Animal 1 June 2014	Animal 5 June 2014
Day							
15	150	150	100	200*	50	0	0
16	100	300*	250	100	200*	1	7
17	200			100	150	12	13
18	250			200	200	51	49
19	100			100	150	65	69
20	300*			100	100	238	89
21	50					151	157
22	150	200	200			149	72
23	50	150	350*			61	40
24						70	59
25						250	200*
26						150	100
27						200	50
28						250*	150
29						150	150
30						200	100
31						250	150
32						150	130
33						50	0
34						120	120
35						98	98
36						89	83
37						100	50
38						37	41
First appearance of eggs (days)	**14**	**14**	**14**	**14**	**14**	**16**	**16**
Peak egg output (days)	**20**	**16**	**23**	**15**	**16**	**28**	**25**
Prepatent period	**17**	**15**	**18.5**	**14.5**	**15**	**22**	**20.5**
Mean prepatent period	**17.5**						

Table 6.2 Egg counts for naïve calves infected with 30,000 *C. oncophora* L3. The timing of peak egg output used to estimate the prepatent period is denoted with an asterisk (*).

	Animal 1 Feb 2012	**Animal 2 Feb 2012**	**Animal 3120 July 2014**	**Animal 4343 July 2014**
Day				
15	850	850	0	0
16	900	1100	0	0
17	550	850	50	150
18	1000*	1550*	0	100
19			200	100
20			100	600
21	600	750		
22	500	850	150	700*
23	500		300	650
24	300	1150	450	450
25	300	900	250	550
26			300	450
27			500*	150
28			200	200
29			250	400
30			100	0
31			0	0
First appearance of eggs (days)	**13**	**13**	**17**	**17**
Peak egg output (days)	**18**	**18**	**27**	**22**
Prepatent period	**15.5**	**15.5**	**22**	**19.5**
Mean prepatent period	**18.1**			

Appendix B: Data used to calculate the correcting factor for host grazing behaviour.

Table 6.3 Data on the vertical distribution of L_3 from various GIN species used for the calculation of the correction factor for host grazing behaviour.

Study	Species	Proportion of L_3 below 5cm	Proportion of L_3 above 5cm
Callinan and Westcott (1986)	*Teladorsagia* and *Trichostrongylus* spp.	0.90	0.10
Callinan and Westcott (1986)	*Teladorsagia* and *Trichostrongylus* spp.	0.86	0.14
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0.00
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.89	0.11
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.87	0.13
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.77	0.23
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.91	0.09
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.85	0.15
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.9	0.1
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.85	0.15
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.94	0.06
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.72	0.28
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.97	0.03
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.44	0.56

Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.83	0.17
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.83	0.17
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.77	0.23
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.68	0.32
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.9	0.1
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.58	0.42
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.73	0.27
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.65	0.35
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	1.00	0
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.75	0.25
Silangwa and Todd (1964)	*Haemonchus placei, Cooperia oncophora* and *Trichostrongylus colubriformis*	0.78	0.22
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.99	0.01
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.64	0.36
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.63	0.37
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.63	0.37
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.62	0.38
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.62	0.38

Crofton (1948)	*Trichostrongylus raetortaeformis*	0.6	0.4
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.61	0.39
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.65	0.35
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.63	0.37
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	0.99	0.01
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Crofton (1948)	*Trichostrongylus raetortaeformis*	1.00	0
Average		**0.85**	**0.14**

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