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Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs

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Summary: Gastrointestinal (GI) nematode control has an important role to play in increasing livestock production from a limited natural resource base and to improve animal health and welfare. In this synthetic review, we identify key research priorities for GI nematode control in farmed ruminants and pigs, to support the development of roadmaps and strategic research agendas by governments, industry and policymakers. These priorities were derived from the DISCONTOOLS gap analysis for nematodes and follow-up discussions within the recently formed Livestock Helminth Research Alliance (LiHRA). In the face of ongoing spread of anthelmintic resistance (AR), we are increasingly faced with a failure of existing control methods against GI nematodes. Effective vaccines against GI nematodes are generally not available, and anthelmintic treatment will therefore remain a cornerstone for their effective control. At the same time, consumers and producers are increasingly concerned with environmental issues associated with chemical parasite control. To address current challenges in GI nematode control, it is crucial to deepen our insights into diverse aspects of epidemiology, AR, host immune mechanisms and the socio-psychological aspects of nematode control. This will enhance the development, and subsequent uptake, of the new diagnostics, vaccines, pharma-/nutraceuticals, control methods and decision support tools required to respond to the spread of AR and the shifting epidemiology of GI nematodes in response to climatic, land-use and farm husbandry changes. More emphasis needs to be placed on the upfront evaluation of the economic value of these innovations as well as the socio-psychological aspects to prioritize research and facilitate uptake of innovations in practice. Finally, targeted regulatory guidance is needed to create an innovation-supportive environment for industries and to accelerate the access to market of new control tools.

KEYWORDS
anthelmintics, control, gastrointestinal nematodes, livestock, parasite, research gaps

1 | INTRODUCTION

Ongoing socio-economic, as well as climatic, changes increasingly emphasize the need for food security (Godfray et al., 2010) and for sustainable livestock production systems that minimize pressure on the environment (Garnett et al., 2013). In this context, animal disease control in general, and gastrointestinal (GI) nematode management in particular, has an important role to play in increasing livestock production to meet future needs of high protein foods from a shrinking natural resource base and to help reduce greenhouse gas emissions from the livestock sector to meet internationally agreed emissions targets (Bartley, Skuce, Zadoks, & MacLeod, 2016; Charlier et al., 2015a).

GI nematode infections are the cause of common, and economically very important, diseases in cattle, small ruminant, pig and poultry production systems around the world. Essentially, all livestock with outdoor access are exposed to these parasites, while some GI nematode species also thrive in pigs and poultry reared indoors. In specific cases, mortality can be high (e.g., haemonchosis in lambs). However, most often GI nematode infections are chronic and associated with hidden subclinical losses such as reduced weight gain, wool growth, milk yields and reproductive performance. Such losses have become increasingly important in the current economic climate, with farmers having to improve production efficiency for the survival of their enterprise (van der Voort et al., 2013).

The control of GI nematode infections in livestock, over the past decades and still today, is primarily based on the preventive or curative use of chemotherapeutics (Vercruysse & Dorny, 1999). However, by way of their inherent genetic diversity, GI nematodes have consistently found ways to circumvent existing control measures. As a consequence, we are currently faced with an escalating spread of anthelmintic resistance (AR) and infection patterns that may be altered by a changing climate, altered land-use and associated farm husbandry changes (Skuce, Morgan, van Dijk, & Mitchell, 2013). It is therefore crucial to (i) understand the mechanisms responsible for the ongoing epidemiological changes; (ii) refine and develop new approaches to safeguard the efficacy of existing control tools; (iii) develop new control tools, including vaccines and efficacious anthelmintic compounds, to maintain the production and welfare standards of livestock production; and (iv) understand human behaviour to see how existing and new tools, and sustainable control approaches, can be implemented effectively. The aims of this review are to identify the research priorities in GI nematode control of farmed ruminants and pigs. Some of the suggested areas of research, such as vaccine and drug development, diagnostics and anthelmintic resistance, also rely on the outcome of basic research in the areas of parasite genomics, transcriptomics, proteomics and metabolomics. These areas have been reviewed and discussed elsewhere (Cantacessi, Campbell, & Gasser, 2012; Cantacessi, Hofmann, Campbell, & Gasser, 2015) and are not the focus of this paper. The current review was built on the Disease & Product analysis conducted by the DISCONTOOLS expert group for nematodes (www.discontools.eu) and on discussions within the recently formed Livestock Helminth Research

Alliance (LiHRA) (Box 1). In the following sections, we will clarify the nematode species involved in this review. Next, we describe recent progress made, and the most critical research gaps in the field, specifically in the areas of pathogenesis, epidemiology, socio-economics, immunology and the main means of prevention, detection and control. This review can be used to build road maps and strategic research and implementation agendas by funders of animal health research, policymakers and other stakeholders at international, European and national level.

2 | KEY SPECIES INVOLVED

Parasitic gastroenteritis (PGE) in European cattle results principally from infections with *Ostertagia ostertagi* in the abomasum and *Cooperia oncophora* in the small intestine. Although the genus *Cooperia* is less pathogenic than *Ostertagia*, these parasite species usually coexist in the same host, with one adding to the pathogenic effect of the other. Immunity also builds up more quickly against *Cooperia* and therefore, in adult cattle, *O. ostertagi* is normally seen as the single most important species. In European sheep and goats, *Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus* spp. and *Nematodirus* spp. are the most pathogenic GI nematode species, contributing significantly to PGE. In domestic pigs, *Ascaris suum* is the most prevalent intestinal species worldwide and is of particular economic importance in fatteners. Other important species are *Trichuris suis* in fatteners and *Oesophagostomum dentatum* in adult pigs (Thamsborg, Nejsum, & Mejer, 2013).

3 | MECHANISMS OF PATHOGENICITY

Pathogenicity varies depending on the nematode genera (species) concerned. *Ostertagia*, *Teladorsagia* and *Trichostrongylus* spp. influence food intake, protein absorption and utilization and can cause diarrhoea, with loss of plasma protein into the gut. *Haemonchus* spp. is a blood-sucking worm, which can cause anaemia. *Nematodirus* spp. induce a hypersensitivity reaction in severely infected parts of the small intestine, followed by a mass shedding of villi, thereby distorting the intestinal water balance and resulting in potentially life threatening diarrhoea. *A. suum* causes nutrient malabsorption, intestinal occlusion, pulmonary dysfunction and predisposes to secondary bacterial infections in the lungs. *Trichuris suis* can cause a haemorrhagic diarrhoea (dysentery), particularly in neonates (Taylor, Coop, & Wall, 2015).

Under certain circumstances, clinical signs can be severe with high mortality rates in mass-invasions of sheep and goats with genera such as *Haemonchus* and *Nematodirus*. However, in general, GI nematode infections are chronic and subclinical, and their main impact is to reduce production efficiency.

The underlying mechanisms for the impact of helminths on production can be divided into three main components: (i) reduced feed intake; (ii) direct tissue damage and decreased functioning of the

BOX 1   The Livestock Helminth Research Alliance (LiHRA)

At the beginning of the 2000s, veterinary parasitologists experienced a general decline in funding opportunities for their discipline (Coles, 2001; Thompson, 2001). This was ascribed to the success of modern anthelmintics, which seemed to have offered a comprehensive solution to the deleterious effects of GI nematode infections, and the greater societal concern over epizootic or zoonotic diseases such as bovine spongiform encephalopathy, which consequently obtained a large share of the available funds for animal disease control and associated research (Coles, 2001). Fifteen years later, the inflection point seems to have been passed. During the difficult years and with support from multidisciplinary EU research programmes (e.g., COST Action CAPARA, EU FP6 PARASOL, EU FP7 GLOWORM, EU FP7 PARAVAC and EU Horizon2020 Paragone) and industry, veterinary parasitologists have assimilated advances in molecular biology, immunology, computer science and epidemiological methodologies. Whereas prevention and vaccination programmes have led to the successful reduction or elimination of several epizootic diseases (Stahl et al., 2005; Sutmoller, Barteling, Olascoaga, & Sumption, 2003), helminth infections have persisted at high levels and elimination is not considered as a realistic option. With the current challenges to food security and sustainable livestock production, the increased emphasis on animal welfare and biological farming stimulating outdoor grazing and the global spread of AR, the importance of GI nematode infections has grown (O'Brien, Scudamore, Charlier, & Delavergne, 2017). There now seems to be an increasing number of funding opportunities, provided the subject area continues to embrace multidisciplinarity and establish strategic research alliances. To support this process, in December 2014, the LiHRA was founded comprising international partners with a recognized expertise in different disciplines applied to helminth (including GI nematode) research. LiHRA unites diverse areas of expertise and, in relation to helminth infection of livestock, it aims to

- Stimulate collaborative research by enabling exchange of ideas and mobility of young researchers;
- Initiate and coordinate research initiatives at the international and national level;
- Facilitate knowledge exchange with the livestock industry and other stakeholders to respond to their needs;
- Respond to global changes that impact on livestock, farming practices and helminth infections and identify areas for future research;
- Foster technology exchange and standardization of diagnostic procedures, clinical trial and monitoring approaches throughout Europe.

At the time of writing, the alliance has 16 member organizations from 10 European countries (www.lihra.eu). Currently, efforts are underway to expand LiHRA to a global scale. Through collaboration, LiHRA aims to become the leading research alliance in the field of livestock helminth infections with the mission to develop sustainable helminth control strategies and promote their implementation by the livestock industry.

affected organs; and (iii) the diversion of energy and protein resources of the host from production towards defence and immune mechanisms. Reduced feed intake is a common feature of all helminth infections, linked with hormonal changes in the host and is thought to be the major mechanism of subclinical production impacts of GI nematodes (Forbes et al., 2009). It has been shown that lactating cows that were pastured under continuous stocking management and treated with an effective anthelmintic, grazed on average 50 min per day longer than their untreated counterparts and this was accompanied by an increase in milk production (Forbes, Huckle, & Gibb, 2004). These effects occurred even at low pasture GI nematode infection levels. There are indications that the reduced appetite may be the result of the increased gastrin levels associated with an increase in abomasal pH, which is, in turn, a result of damage to the parietal cells (Coop & Kyriazakis, 1999). However, the exact neuroendocrine mechanisms of parasite-induced inappetence

are probably more complex and yet to be unravelled. With today's increased access to electronic devices and sensors for automatic registration of animal movements, grazing behaviour, body condition and gastrointestinal fluid dynamics, more focus in this area may discover fundamental insights in how nematodes affect animal health, welfare and productivity (Szyszka, Tolkamp, Edwards, & Kyriazakis, 2013).

Besides reduced appetite, the energy requirements of the immune response are an important drain on the finite energy sources of all infected animals and, arguably, ruminants in particular. In sheep, it has been estimated that the maintenance of immunity to nematode parasites incurs a 15% loss of productivity due the diversion of nutrients away from productive functions to the immune system (Greer, 2008). Immuno-suppressive corticosteroid treatment of *T. circumcincta*-infected lambs results in higher faecal egg counts (FECs) and worm burdens, but improves energy utilization and

performance as compared to non-immunosuppressed infected control lambs (Greer et al., 2008). Dever, Kahn, Doyle, and Walkden-Brown (2016) found that the immunological response of grazing meat lambs to *T. colubriformis* infection accounted for 75% of the overall cost of infection (with the majority of this cost occurring during the first 35 days of infection). These examples confirm that the host's immunological response to nematode infection can be the major component of production loss, at least in growing sheep. In cattle, hypersensitivity reactions to even small numbers of developing larvae have been described, but the importance of the immune response as a cause of production losses has not been studied to date (Berghen, Hilderson, Vercruysse, & Dorny, 1993).

4 | EPIDEMIOLOGY

Infection pressure with GI nematodes in grazing animals varies through the year as a function of climate and farm management. In most farming systems, therefore, seasonal patterns of infection emerge, allowing standardized control practices based on regular anthelmintic application on a fixed calendar, and which can be communicated to farmers in simple, practical, terms (e.g., Hawkins, 1993). Recently, changes in climate, land-use and farm management have posed challenges to the established control programmes. For example, in temperature regions, prolonged grazing seasons in warmer autumn-spring conditions present new opportunities for transmission of parasites (Phelan, Morgan, Rose, Grant, & O'Kiely, 2016), while hot dry summers can drive biphasic peaks in infective stage development (Rose et al., 2016). In sheep, there is already evidence that changes in the dominant seasonality of GI nematode disease are linked to climate change (Van Dijk, David, Baird, & Morgan, 2008). Control programmes therefore need to be re-evaluated and adapted to maintain their efficacy.

Devising new control programmes and strategies is complex and must take into account effects of multiple interactions on parasite populations, which may be conflicting and different for each worm species. Therefore, for each adaptive change in management, the consequences on the whole system need to be considered before intervening (Gauly et al., 2013). Mathematical transmission models that simulate disease dynamics and host responses are therefore key to improving our understanding of parasite epidemiology under rapidly changing conditions, and predicting optimal responses *in silico*, before making practical recommendations or collecting detailed empirical data. Recent progress allows us to model the impacts of climate change (Rose, Wang, van Dijk, & Morgan, 2015a) combined with novel control strategies (Berk, Laurenson, Forbes, & Kyriazakis, 2016) on nematode epidemiology. Once isolated mechanisms are understood, mathematical models can be integrated within the whole system of study, and can explore mitigation of climate-mediated increases in infection by targeted management (Morgan & Wall, 2009). The poor quantitative understanding of acquired immunity mechanisms remains a major bottleneck for further elaboration of these models for GI nematodes. Designing lifetime management strategies, for instance, could trade growth performance of animals off against longer term resistance (Claerebout, Vercruysse, Donry, Demeulenaere, & Dereu, 1998), but in order for economic optimization to be possible, a quantitative understanding of the trade-offs is needed. Furthermore, attention must be given to the implementation of model predictions on farms and integration with farmer decision systems. To date, scientific advances in modelling nematode systems have improved general epidemiological understanding but arguably made little difference to practical parasite control on individual farms. A wider skill-set and greater commercial sophistication will be necessary to lever the potential impacts of these models more effectively (Verschave, Charlier, Rose, Claerebout, & Morgan, 2016).

Until now, most studies designed to develop new control approaches have focused on single nematode infections. In reality, multiple parasite taxa (including GI nematodes, lungworms, protozoa and trematodes), as well as bacterial and viral infections, often occur together. Multiparasitism can be the result of common drivers ("risk factors") for infection (Musella et al., 2014) or by direct and indirect interactions or synergies between different pathogens (e.g., by altering immunological responses; Salgame, Yap, & Gause, 2013). These interactions, and their implications for disease outcomes and control strategies, have remained largely unexplored to date. The advent of multiplexed and next-generation sequencing (NGS) diagnostic technologies now allows us to more easily characterize the whole nematode community present in a single host (Avramenko et al., 2015). This will help us to understand how parasite communities as a whole respond to human intervention and environmental changes, rather than only isolated species or genera.

Poorly understood interactions at the level of the host animal also limit our current understanding of nematode epidemiology and the dissemination of AR, for example, the role of livestock movement between farms and to/from livestock markets (Skuce, Stenhouse, Jackson, Hypsa, & Gilleard, 2010) and the dissemination of resistant parasites by wildlife reservoirs, for example, BZ-R *H. contortus* by deer (Chintoan-Uta, Morgan, Skuce, & Coles, 2014). This will require further advancement in the field of molecular epidemiology and analysis of population genetic structure, potentially at continental scale (e.g., Blouin, Yowell, Courtney, & Dame, 1995; Gilleard & Redman, 2016), as high levels of animal movements have been recorded, for example, across Europe (Hardstaff, Häsler, & Rushton, 2015).

Finally, it will be important to complement predictive modelling capability with strong empirical research, if we are to have confidence in the conclusions of computer models. Charlier et al. (2016) assessed the trends of GI nematode infections of cattle over an 8-year period in a large cohort of dairy farms. The observed trends showed marked differences compared to the long-term predictions from mathematical models, suggesting that management or other hidden factors were insufficiently accounted for in these models. The authors recommended the establishment of a network of sentinel farms that should be monitored over time using bulk tank milk samples. A recent study showed that such a monitoring approach is also an effective decision support tool as it led to year-on-year

reductions in the study farms' infection status, at least for *F. hepatica* (Munita et al., 2016).

5 | SOCIO-ECONOMICS

The impact of GI nematodes on productivity depends on the host species, its geographical location and physiological status and will further be largely dependent on the degree to which a farmer can counteract infection-induced energetic losses by the provision of protein-rich diets (Kyriazakis & Houdijk, 2006). Quantified production impacts in ruminants have recently been reviewed by Charlier, van der Voort, Kenyon, Skuce, & Vercruysse (2014a) and Mavrot, Hertzberg, and Torgerson (2015), and for nematodes of pigs by Thamsborg et al. (2013). However, there are also several studies that failed to show any impact of infection in pigs, perhaps because of the lack of good diagnostic tools to detect the presence of infection (Vlaminck, Levecke, Vercruysse, & Geldhof, 2014) and the temporary effect on productivity in infected animals. Nevertheless, even if the production impact of subclinical nematode infections may be subtle, in current economic climates with small profit margins for farmers, GI nematode infection has been shown to exert a disproportionate impact on the economic profitability of farms (Van Meensel et al., 2010). Whereas an increasing amount of data are being generated for the direct production impacts of GI nematode infections, more emphasis should now be given to the production and economic impacts of AR.

In contrast to transboundary or zoonotic diseases, where control measures are mostly taken collectively by policy interventions, so far the control of GI nematode infections has remained the individual responsibility of the farmer. Given the present situation with spreading AR (Gasbarre, 2014; Geurden et al., 2015; Rose et al., 2015b) and the low implementation of "best practice" parasite management programmes (McArthur & Reinemeyer, 2014), a discussion as to whether policy-driven intervention is now required is warranted. The availability of cheap generic anthelmintics favours the indiscriminate use of the products at the expense of veterinary consultation and the use of diagnostics to inform anthelmintic treatment decisions. There is agreement between scientists that a major mentality shift will be required to put "best management" advice widely into practice. Recent examples on the development of best practice recommendations using a stakeholder-driven approach are the SCOPS and COWS initiatives in the UK (Taylor, 2012). However, how to best achieve the implementation of such recommendations, whether it is by socio-psychological insights into the farmer's mindset and adapted communication strategies (Vande Velde et al., 2015) or by stricter regulation as was done in the Nordic countries (Thamsborg, Roepstorff, & Larsen, 1999), is still being debated.

Given that the costs of parasite control measures are typically borne by the farmer, there is a pivotal role for the development of models and computational tools to assess the farm-level economic impact of nematode as well as other infections. Several models have already been developed, but they need further translation and support to evolve from research to practice (Charlier et al., 2015b). In addition, these models are mostly restricted to dairy cattle farms and need to be extended to include beef, sheep, goat and pig production systems. The reliability and utility of the models will depend on progressive insights into production impacts, diagnostic tools to measure both infection level and production impact, the effects of co-infections and multiparasitism (Viney & Graham, 2013). Models further need to be able to quantify the trade-off between short-term economic benefits of intense anthelmintic treatments and the loss of efficacy over several years (Laurenson, Bishop, Forbes, & Kyriazakis, 2013; Learmount et al., 2016).

6 | IMMUNE RESPONSE TO INFECTION

In general, cattle develop a strong T helper-2 type immune response following a GI nematode infection. However, whether and how this type of response actually protects the hosts against further worm infections is still a matter of debate, especially for *O. ostertagi* infections in cattle (Rinaldi & Geldhof, 2012). Despite the induction of a strong host immune response within the first few weeks following an infection, animals typically remain susceptible to new infections for months or even years on farms (Gasbarre, 1997). The immune response triggered is characterized by a strong proliferation of lymphocytes in the local draining lymph nodes, eosinophil and mast cell infiltration in the abomasal mucosa, increased levels of parasite-specific IgG, IgM and IgA and the production of IL4, IL5 and IL10 (reviewed by Rinaldi & Geldhof, 2012; Mihi et al., 2014). A similar type of immune response has also been observed following *C. oncophora* infection with the production of parasite-specific IgA and IgG1 (Kanobana, Vervelde, Van Der Veer, Eysker, & Ploeger, 2001) and an eosinophil influx at the site of infection (Kanobana, Koets, Bakker, Ploeger, & Vervelde, 2003; Kanobana, Ploeger, & Vervelde, 2002). However, in contrast to *O. ostertagi* infection, naturally acquired immune protection against *C. oncophora* typically occurs within the first grazing season. The response is visible in worm physiology in terms of stunted growth and reduced fecundity of the female worms.

In pigs, *A. suum* and *T. suis* generally provoke strong protective immunity. During primary *A. suum* infections, the majority of larvae are expelled from the intestine by the host from around 17–24 days post-infection (Roepstorff, Eriksen, Slotved, & Nansen, 1997), and with repeated exposure pigs develop "pre-hepatic" immunity which results in larvae being unable to penetrate the caecum to begin the migratory phase to the liver and hence are expelled within 24 hr of ingestion (Urban, Alizadeh, & Romanowski, 1988). Similarly, *T. suis* larvae are expelled by the host in a self-cure reaction beginning around 63 days following a primary infection (Kringel & Roepstorff, 2006). However, particularly for *A. suum*, a small residual adult population may develop, and a small number of animals will continue to harbour large adult worm burdens which contribute markedly to the contamination of the environment with infective eggs (Nejsum et al., 2009). The immune-reactive mechanisms appear to be similar to

other helminth infections, namely a Th2-biased response characterized by eosinophilia, mastocytosis and increased gut permeability and decreased transit time (Kringel, Iburg, Dawson, Aasted, & Roepstorff, 2006; Masure et al., 2013). *Oesophagostomum dentatum* differs in that the Th2-response is markedly delayed and/or lower in intensity (Andreasen et al., 2015). Interestingly, *O. dentatum* infections are usually chronic in nature and tend to accumulate over time, and unlike, *A. suum* and *T. suis* where young animals are most at risk of infection, *O. dentatum* prevalence is higher in older animals such as sows and boars (Roepstorff et al., 1998).

For all host–parasite relationships described above, several immune parameters (antibodies, immune cells, etc.) have been shown to be correlated with the development of acquired immunity. However, experimental evidence for a causal relationship between these immune parameters and protection is still largely missing. One of the reasons for this is the difficulty of performing functional immunological experiments in large animal species. In addition to the lack of knowledge on the actual effector mechanisms themselves, many aspects of the early stages of the immune response, for example, molecular pattern recognition, glycosylation of antigens but also the cells and pathways involved in this process, are largely unknown. In summary, the differences in immune responses witnessed to different worm, and within different host, species, as well as the non-sterile nature of derived immunity and the practical non-interpretability of immune parameters poses potential threats to vaccine development.

7 | MAIN MEANS OF DETECTION, PREVENTION AND CONTROL

7.1 | Diagnostics

7.1.1 | Recent developments in diagnostics

The methods currently used for diagnosis and monitoring of nematode infections, including assessment of drug efficacy, have changed little over the past decades. Current diagnostics typically involve performing FECs to measure infection intensity, occasionally followed by faecal culture with species identity confirmed by larval morphology/morphometric analysis or by conventional/real-time PCR (Avramenko et al., 2015). These approaches are low-throughput in nature, time-consuming and thus expensive. As a result, parasitological diagnosis in veterinary practice is not routinely carried out. Considerable efforts have been recently made to improve the diagnostic performance (e.g., analytic sensitivity, precision and accuracy) and technical performance (e.g., ease of use, cost, user safety, timing) of FEC techniques. Recently developed tools such as FLOTAC (Cringoli, Rinaldi, Maurelli, & Utzinger, 2010), Mini-FLOTAC (Cringoli, Rinaldi, Albonico, Bergquist, & Utzinger, 2013) and FECPAK (www.fecpak.com) have provided alternative methodologies to determine FEC with increased sensitivity and allowing detection of smaller reductions in anthelmintic efficacy (Levecke, Dobson, Speybroeck, Vercruysse, & Charlier, 2012). To increase user-friendliness, portable kits, such as FECPAK^{G2} and Mini-FLOTAC, are now available to provide "on-farm" methods of FEC to make rapid decisions on the need to treat or to determine whether anthelmintics are effective (Cringoli et al., 2013).

Besides coprological diagnosis, methods are available to diagnose *O. ostertagi* infection in cattle by measuring pepsinogen concentration in serum or antibody levels in serum or milk (Charlier et al., 2014b). There is a need to identify additional biomarkers, which can be used in future diagnostic assays for this and other species. Current progress in genomic resources for nematodes, combined with advances in proteomic and metabolomic technologies, is making this increasingly feasible (Cantacessi et al., 2015). One example of a recent advancement is the development of a microbead-based multiplex assay for the simultaneous detection of antibodies directed against *C. oncophora*, *F. hepatica* and the bovine lungworm *Dictyocaulus viviparus*; these now need to be extended to include the detection of morbidity markers (Karanikola et al., 2015). Rapid DNA-based diagnostic tests such as multiplex tandem PCR (MT-PCR) (Roeber et al., 2012), loop-mediated Isothermal amplification (LAMP) methodologies (Melville et al., 2014) and deep sequencing of the ITS-2 rDNA (Avramenko et al., 2015) are also under development. Such technologies are expected to lead to increased sensitivity and specificity as well as more accurate quantification of whole parasitic nematode communities instead of single species. Parallel efforts in pigs include a new serological detection technique to measure the exposure of fattening pigs to *A. suum* (Vlaminck et al., 2012), which needs to be correlated with measures of animal productivity.

The application and further improvement of these new diagnostic tools will undoubtedly lead to new insights into how parasites respond to control strategies, what impact they have on animal productivity, and how they interact with their host and with each other. Finally, a new impetus is needed towards the development of penside diagnostics, a field that has shown recent progress through automated FEC methods, smartphone image capture and computational image analysis that can be used on farm and that could be made more user friendly (Slusarewicz et al., 2016).

7.1.2 | Diagnostics for targeted (selective) treatments

Two important concepts were introduced to study and promote the sustainable use of anthelmintics (Kenyon & Jackson, 2012): targeted treatment (TT), where the whole flock/herd is treated based on knowledge of the risk, or parameters that quantify the mean level of infection, and targeted selective treatment (TST), where only individual animals within the grazing group are treated, based on a single treatment indicator, or a combination of indicators. These can consist of parasitological parameters (e.g., FEC), production parameters (e.g., weight gain, body condition scoring) or morbidity parameters (e.g., serum pepsinogen concentration, FAMACHA©, breech soiling score). The aim of the TT and TST approaches is to effectively control nematode-induced production impacts while preserving anthelmintic efficacy by maintaining a pool of untreated parasites

"*in refugia*" within hosts and on pasture, which can complete their life cycle and thereby pass on susceptibility-associated genes to the next generation (Van Wyk, 2001). It is now widely accepted that TT/TST can reduce anthelmintic use, with some studies showing that these approaches can also slow the development of AR (Kenyon et al., 2013; Waghorn, Leathwick, Miller, & Atkinson, 2008).

An important limitation for the development and validation of TT/TST strategies, as well as associated epidemiological models, is the lack of validated tools to quantify the parasite population on pasture. Current methods use pasture larval counts in conjunction with species identification, but are labour intensive and suffer from low reproducibility (Verschave, Levecke, Duchateau, Vercruysse, & Charlier, 2015).

Each specific TT/TST strategy must be adjusted to local farming conditions. Important challenges are to define the (combination of) diagnostic marker(s) that can be used for identification of the groups or individuals that need to be treated, and to determine treatment thresholds (e.g., Höglund, Dahlström, Sollenberg, & Hessle, 2013; Merlin et al., 2016). To further convince farmers of the benefits of implementing these approaches, on-farm studies are required to confirm and extend existing empirical findings. Such trials require a long-term working relationship between farmers and researchers and would benefit from the availability of molecular AR markers, to provide direct evidence that TT/TST slows the development of AR.

Many TT/TST studies have used indicators of parasite burden (e.g., FEC), immunological (e.g., antibody levels in milk) or patho-physiological indicators (e.g., FAMACHA) for treatment, which do not necessarily correlate to negative production effects. Tools that quantify the consequences of infection, rather than the level of GI nematode infection per se, and which can facilitate cost/benefit analyses of the proposed interventions, therefore need to be developed. Recent progress in this area includes the establishment of the links between the results of several diagnostic tests, and production impact or production responses after anthelmintic treatment (for review, see Charlier et al., 2014a). However, much work remains to be done, especially on the implementation of the new diagnostics described above, which could lead to more cost-effective diagnosis.

To enable such implementation as part of routine farm management, user friendly, cost-effective decision support tools are required. These need to be implemented as a "one-stop shop" where support can be found for multiple diseases and production-limiting conditions in the same place, so that farmers need to use only a single application. These decision support systems will complement, or even act synergistically with, precision livestock farming approaches. The advent of Internet-of-Things (IoT) technologies combined with ongoing sensor miniaturization is unlocking new opportunities to collect and interpret large amounts of animal information, such as location, movement, sound, temperature, breath and gastrointestinal tract motility (Berckmans, 2014). These advances make remote monitoring of extensively grazed animals a reality and assist farm management at a time when the labour available on farms continues to decline (Rutten, Velthuis, Steeneveld, & Hogeveen, 2013).

7.1.3 | Diagnostics for Anthelminitic resistance

The current *de-facto* test for AR detection in all drug classes is the *in vivo* "faecal egg count reduction test" (FECRT) (Coles et al., 2006). This test requires two samples obtained 7–14 days apart and from at least 10 animals. Consequently, the FECRT is slow, labour intensive and expensive to perform. This limits its application in the field and the geographical range and number of farms included in surveys of the extent of AR. Other limitations of the FECRT include the effects of temporary suppression of egg production by resistant worms after ML treatment, leading to false negative results. The FEC method used also affects the FECRT, requiring careful sampling design and test interpretation (De Graef, Claerebout, & Geldhof, 2013). These limitations increase uncertainty around FECRT results, and meaningful comparable information on the distribution and extent of AR is consequently lacking (Rose et al., 2015b).

Promising results have been obtained in pilot studies using pooled faecal samples to decrease the work load and cost of conducting FECRT (George, Paras, Howell, & Kaplan, 2017; Kenyon et al., 2016; Rinaldi et al., 2014). Recently, the feasibility has been demonstrated of producing portable FEC kits combined with a mobile phone application for image capture and specific worm egg quantification and identification (Slusarewicz et al., 2016). Using such labour saving novel tools to detect AR would enable larger international surveys to map the distribution and extent of AR in GI nematodes of ruminants throughout Europe and to study associated risk factors, as well as to make assessments of drug efficacy more accessible to individual farms.

Beyond the FECRT, several *in vitro* assays have been developed for the assessment of anthelmintic susceptibility in GI nematode populations (for review, see Demeler, Schein, & von Samson-Himmelstjerna, 2012a). Compared with the FECRT, these assays all have the advantage of requiring less effort in the field (only one faecal sampling, with no anthelmintic treatment required). The tests include the egg-hatch-inhibition assay (EHA) to evaluate the effect of BZs on the hatching of ruminant GI strongyle eggs. The larval-development-inhibition assay (LDA) was developed in the 1990s for the evaluation of the susceptibility of sheep GI nematodes to BZs, tetrahydropyrimidines and imidazothiazoles, and macrocyclic lactones (MLs). More recently, it was successfully used to test the effect of MLs and levamisole (LEV) against GI nematodes of cattle (Demeler, Küttler, & von Samson-Himmelstjerna, 2010). Another test is the larval-migration-inhibition assay (LMA) that can be employed to study the effect of MLs *in vitro* (Demeler, Kleinschmidt, Küttler, Koopmann, & von Samson-Himmelstjerna, 2012b). Whereas the tests typically require parasite eggs or larval stages, recently also adult parasites have been successfully used in *in vitro* assays (Demeler, von Samson-Himmelstjerna, & Sangster, 2014). Despite the availability of these *in vitro* tests, they are not implemented in routine diagnostic laboratory procedures. This may be due to requirements for specific laboratory equipment and technical expertise, which are not present in all state or commercial diagnostic laboratories, and lack of demand from farmers unconvinced of the necessity to test for AR.

Molecular approaches also promise to be of practical use for the evaluation of anthelmintic susceptibility. However, complex processes are involved in the development of AR at the cellular and genetic level and this has, thus far, prevented the development of routine molecular tests to detect AR (Kotze et al., 2014). The one exception to this situation is resistance to the benzimidazoles, BZs (BZ-R), which is associated in trichostrongylid nematodes with the accumulation of specific mutations in the parasites' beta-tubulin gene (Wolstenholme, Fairweather, Prichard, von Samson-Himmelstjerna, & Sangster, 2004). Based on this knowledge, sensitive and field applicable tests for the analysis of BZ-R in ruminant parasites have been recently developed (Demeler et al., 2013; Ramünke et al., 2016). However, respective tests for other drug classes, like the MLs, arguably the most important drug class, are lacking and the available BZ-R tests require species-specific PCR and analysis. Accordingly, molecular assessment of anthelmintic susceptibility is still not a component of routine diagnosis in the field and further improvements are required, both concerning the spectrum of drug class for which meaningful molecular tests are available, and the cost associated with testing.

7.2 | Therapeutics

Since the mid-1960s, the control of GI nematodes in livestock has heavily relied on anthelmintics (Coles, 2002). The three major anthelmintic families are (i) the BZ, including albendazole, which is still widely used in sheep in Europe, (ii) imidazothiazoles and tetrahydropyrimidines (which include levamisole (LEV) and pyrantel (PYR)) and (iii) macrocyclic lactones (ML) including ivermectin and moxidectin. In some countries, two new actives have been licensed and launched onto the sheep market: the amino-acetonitrile derivatives (AAD), that is, monepantel, and the spiroindoles (SI), of which derquantel is used in a dual-active product with abamectin. Nematodes in pigs are also mainly controlled by application of anthelmintics, particularly BZ and ML.

At the time of first registration, all anthelmintics used in livestock were very effective, typically reducing susceptible worm burdens by at least 90% (BZ, PYR & LEV) up to 99% (ML, AAD, SI + ML) (Coles et al., 2006). Despite progress in the development of parasite vaccines and other novel control methods (see below), anthelmintics will remain vital for the control of GI nematodes in the foreseeable future, either alone or in combination with other novel control methods. Possible drawbacks of the use of anthelmintics include: the increasing development and spread of AR; possible reduced or delayed development of natural immunity against nematodes; consumer concerns regarding drug residues in food products, for example, meat and milk; and concerns regarding the impact of these products when excreted into the environment.

The escalating spread of AR is considered the single biggest threat to sustainable nematode control and, if not acted upon, may result in major economic losses for the livestock industries. AR is now widespread in all the major GI nematodes of sheep and is an emerging problem in cattle nematodes globally, mostly involving ML resistant *Cooperia* spp. (Sutherland & Leathwick, 2011).

Anthelmintic actives with a new mode of action, either stand alone or in combination, or novel combinations of actives against GI nematodes belonging to the currently available classes would greatly assist in managing AR (Martin et al., 2015; Smith, 2014). In this respect, differentiation needs to be made between combination products incorporating two or more constituent actives to expand efficacy against helminth parasites belonging to a different phylum (e.g., GI nematodes and liver fluke), and combinations of two or more actives targeting only GI nematodes. The former are developed based more on a combination of commercial interest and convenience for the end-user and often ignore different risk factors and optimal timing of treatment for the targeted helminth species; the latter can increase the efficacy of the anthelmintic against resistant nematode populations and potentially postpone the development of AR against single compounds (Geary et al., 2012). Guidelines on the requirements for combination products targeting nematode infections are available (Geary et al., 2012) but, in Europe, these have not yet been investigated to create a better regulatory environment for the development of such combination or multi-active products. A recent reflection paper on AR from the European Medicines Agency (Anonymous, 2016) recommended to further explore the benefits and risks in relation to resistance development associated with the use of multi-active anthelmintics. In Australasia and South America, multiple active products (in this context, meaning a product containing two or more different anthelmintic classes with activity against the same parasite spectrum) are commonplace but, in Europe, only one such product has been licensed (SI + ML for use in sheep). Current opinion is that the use of such products, under the correct conditions, can slow down selection for resistance (Bartram, Leathwick, Taylor, Geurden, & Maeder, 2012; Leathwick & Besier, 2014). Some concerns exist that, if used incorrectly, resistance will develop to multiple actives at the same time (Besier, 2007). Recently, it was found that if the use of multiple active anthelmintics is combined with "best practice parasite management," based on avoiding overuse of anthelmintics, minimizing nematode challenge to susceptible animals, and maintaining a nematode population *in refugia*, resistant populations may even be reversed towards susceptibility (Leathwick, Ganesh, & Waghorn, 2015). However, implementation of best practice management brings us back to the need for and gaps in development of diagnostics and effective knowledge transfer to end-users.

The question of whether AR carries a fitness cost to the parasite (Bartley, Devin, Nath, & Morrison, 2015; Leathwick, 2013) in the field and how this can be exploited to develop strategies to lead to a reversion to susceptibility are important for the long-term sustainability of the currently available actives.

Obviously, there is a clear need to generate and analyse field evidence to underpin recommendations for targeted drug use in the interests of sustainable efficacy, and to understand the economic implications of such approaches. From an industry perspective, any future anthelmintic product (mono-active or multiple active) will have to be framed within a management plan. In addition, industry is faced with the question of how a future product can compete with

the multiple generic products (with reduced efficacy) that are already on the market. Therefore, a regulatory environment that promotes best practice parasite management recommendations and prohibits the use of anthelmintics with low efficacy may be key to stimulate future innovation in the field of therapeutics.

7.3 | Vaccines

The limitations of control through anthelmintics have prompted research into nematode vaccine development. Ideally, vaccines would provide durable protection, with no associated chemical residue issues. However, the only vaccine against GI nematodes currently on the market is a subunit vaccine for *Haemonchus contortus* in sheep, available in Australia (Barbervax) and South Africa (Wirevax) that needs to be administered at monthly intervals to maintain protection.

Experimental vaccines against other GI nematodes in livestock are at various stages of development (reviewed by Matthews, Geldhof, Tzelos, & Claerebout, 2016). A key starting aspiration for a scalable vaccine is the identification of worm antigens that consistently give protection in vaccine trials. Several antigens from *H. contortus* have been proved successful when purified from adult worm gut extracts, including a microsomal aminopeptidase (H11) and a galactose-containing glycoprotein complex (H-gal-GP), which are the main components of the commercial Barbervax vaccine. A low molecular weight protein from adult worm somatic extracts was also protective in consecutive vaccination experiments (Alunda, Angulo-Cubillan, & Cuquerella, 2003; Dominguez-Torano et al., 2000; Fawzi, Gonzalez-Sanchez, Corral, Cuquerella, & Alunda, 2014). In cattle, vaccination with native activation-associated secreted proteins (ASP) from adult *O. ostertagi* and *C. oncophora* repeatedly gave a good reduction in FEC (Geldhof et al., 2003; Vlaminck, Borloo, Vercruysse, Geldhof, & Claerebout, 2015). In pigs, vaccination with *Ascaris suum* haemoglobin failed to induce protection (Vlaminck et al., 2011). To our knowledge, no other native *A. suum* proteins have been tested in pigs.

To upscale vaccine production and to reduce production costs and batch-to-batch variability, most commercial vaccines would require recombinant vaccine antigens. However, obtaining acceptable protection levels with recombinant antigens has proven difficult. Several recombinant vaccine antigens, expressed in *Escherichia coli*, insect cells, *Pichia pastoris* or the free-living nematode *Caenorhabditis elegans*, failed to confer protection in vaccine trials (Cachat, Newlands, Ekoja, McAllister, & Smith, 2010; Geldhof, Meyvis, Vercruysse, & Claerebout, 2008; Roberts et al., 2013; Vlaminck et al., 2011). Current research is focusing on differences in protein folding or secondary modifications, such as glycosylation, between the native and recombinant proteins as possible reasons for the lack of protection (Matthews et al., 2016). Recently, a number of recombinant vaccines showed promising results. The *H. contortus* ES antigen Hc23, expressed in *E. coli*, protected lambs against an artificial challenge infection (Fawzi, Gonzalez-Sanchez, Corral, Alunda, & Cuquerella, 2015). A vaccine "cocktail" comprised of eight recombinant proteins from *T. circumcincta*, expressed in *E. coli* and *P. pastoris*, protected

lambs against a trickle challenge infection (Nisbet et al., 2013) and reduced faecal egg output in pregnant ewes (Nisbet et al., 2016).

To develop successful vaccines, effective immune responses must be stimulated for an appropriate length of time using easy-to-use delivery methods. A straightforward approach to stimulate a mucosal immune response is to deliver the vaccine antigen directly onto the mucosal surface. Attempts to immunize sheep by delivering antigen directly to the intestinal mucosa showed variable results (Jacobs, Wiltshire, Ashman, & Meeusen, 1999; McClure, 2009). In mice, several recombinant low molecular weight antigens from *A. suum* were reported to induce protective immune responses when administered intranasally, but protection in pigs was only confirmed for one 16 kDa antigen (Tsuji et al., 2004). In ruminants, the most practical method to deliver vaccines is via systemic (intramuscular or subcutaneous) routes and most trials thus far have tested vaccines in this format. Aligned with the route of delivery, adjuvants play an important role in inducing an effective immune response and the choice of adjuvant has shown to be crucial to obtain protection. The saponin adjuvant Quil A has been successfully used in combination with *O. ostertagi* and *C. oncophora* ASPs (Geldhof et al., 2003; Vlaminck et al., 2015) and with the protective *T. circumcincta* antigen cocktail (Nisbet et al., 2013, 2016), while *O. ostertagi* ASP combined with aluminium hydroxide conferred no protection (Geldhof et al., 2004). In contrast, immunization of sheep with HcsL3 of *H. contortus* in combination with aluminium hydroxide gave a significant reduction in FEC, while protection was abolished when the same antigen was used in combination with Quil A (Jacobs et al., 1999; Piedrafita et al., 2013). As adjuvants can steer the immune response to Th1 (Quil A) or Th2 (aluminium hydroxide), these observations suggest that a protective vaccine-induced immune response may be different for different parasites and/or antigens, even within the same host species. Improved knowledge of the immune mechanisms associated with vaccine-induced protection would provide valuable information to improve antigen delivery and choice of adjuvants.

Although there is clearly room for improvement in (recombinant) antigen production and delivery, it is less clear how much improvement is needed for a commercially viable vaccine. Little information is available on how long a vaccine should protect livestock and what levels of protection would be sufficient to prevent disease and production losses. The levels of efficacy required will vary among nematode species and between regions, depending on parasite epidemiology and local farm management practices. For example, it has been suggested that a reduction of cumulative FEC by around 60% during the first 2 months after turnout would sufficiently reduce pasture infection levels to protect young stock against *O. ostertagi* and *C. oncophora* until the end of the grazing season (Claerebout, Knox, & Vercruysse, 2003). However, this hypothesis is based on the assumption that a typical first grazing season in Western Europe lasts for about 6 months, and vaccine efficacy requirements are likely to be different in regions with continuous grazing throughout the year, such as parts of South America and New Zealand (Matthews et al., 2016). Vaccine efficacy requirements may also be different in calves or lambs that co-graze with their dams. In

contrast to *O. ostertagi*, fecundity of *H. contortus* is not regulated by the intensity or duration of the infection, and there is a good correlation between total daily FECs and the mature female worm burden (Anderson & May, 1985). Therefore, to reduce FEC, vaccine-induced immunity should prevent establishment of infective larvae or kill established worms (Claerebout et al., 2003). Moreover, future vaccines could combine antigens from different parasite species (or other pathogens) and vaccination may be combined with other parasite control measures, including anthelmintic treatments.

For logistical, financial and animal welfare reasons, it will be practically impossible to test all possible scenarios by vaccine trials in the field. Modelling vaccine efficacy could be a valuable tool to help define useful levels of protection and to model integrated use of vaccines with other parasite control measures. A model simulating the effect of vaccines against larval stages or adult *H. contortus* in sheep has been developed (Meeusen & Maddox, 1999), but a threshold for protection needed to protect animals from acquiring harmful burdens during the entire grazing season has not been determined. More research needs to be undertaken in this area, as information on the required vaccine efficacy will also be important for registration purposes. At present, regulatory authorities are not familiar with registration of helminth vaccines. As it is unlikely that any vaccine will obtain efficacy levels that are comparable with those of modern anthelmintics (or vaccines against viruses and bacteria), regulatory authorities will need to be informed about thresholds for duration and level of protection that are sufficient to reduce environmental contamination to a level that does not interfere with animal welfare and productivity. These are likely to be dependent on the management and climatic context.

7.4 | Bioactive forages

Bioactive forages, used as part of the diet, can deliver both anthelmintic and nutritional benefits due to the presence of plant secondary metabolites (PSM). As such, they form part of the concepts of nutraceuticals (Hoste et al., 2015), although some may eventually be developed as stand-alone drugs. Legumes containing condensed tannins (CT) and polyphenols (e.g., sainfoin, *Sericea lespedeza*) represent some of the widely studied models of bioactive forages in nematode control. Direct anthelmintic properties of bioactive forages have been confirmed in vitro and in vivo both in small ruminants and cattle (Hoste et al., 2015; Pena-Espinoza, Thamsborg, Desrues, Hansen, & Enemark, 2016). CT have also shown strong *in vitro* activity against GI nematodes of pigs (Williams et al., 2014), but *in vivo* activity has yet to be confirmed.

Potential bioactive forages are found worldwide and their exploitation as nutraceuticals may have generic implications to improve the control of GI nematodes in ruminants globally. This wide distribution has also led to the exploration of non-conventional tannin containing resources such as agro-industrial by-products (Hoste et al., 2015).

There are great variations between different studies investigating the anthelmintic properties of bioactive forages. These are mostly explained by environmental, genetic and technological factors (e.g., harvest times, conservation method, storage conditions) leading to variations in the content and quality of the PSM. Both basic research and applied research are needed to exploit the use of bioactive forages as a reliable method of nematode control and to develop sustainable business cases for its use. First, further research is required on the mode of action of different classes of PSMs against the different GI nematode species and life-cycle stages: identification of the active compounds, how the PSMs interact with the GI nematode structures or molecules, and how quickly nematodes will develop resistance against these natural products (Pena-Espinoza et al., 2016). Tannin-rich feeds have also been shown to improve the immune response to GI nematodes in sheep (Ramírez-Restrepo et al., 2010); however, it is not clear whether this represents an improvement in protein supply to the small intestine or a direct immuno-stimulatory effect, such as activation of innate immune cells (Williams et al., 2016). Second, the pharmacokinetics, distribution and interaction of different PSM in the host need to be investigated. Synergy has been reported *in vitro* between CT and both flavonoid monomers (Klongsiriwet et al., 2015) and aldehydes (Ropiak et al., 2016), but there are also reports of unfavourable interactions between CT and other plant compounds {Arias et al., 2013), suggesting that complex interactions exist between the diverse plant compounds found in forages, warranting further studies. Likewise, the interactions between CT and anthelmintics need to be better defined, with reports of both an enhancement (Hansen et al., 2016) as well as an inhibition of drug activity (Gaudin et al., 2016).

Gaps in applied research include the development of simple methods to quantify the level of PSM in rations before on-farm use (e.g., through near-infra-red technologies to measure the amount and type of CT in crops), practical delivery methods and the feasibility of using PSM in monogastric livestock systems, where the use of forages is less common, such as through feed additives based on agro-industrial waste products.

7.5 | Biological control through nematode-destroying fungi

Biological control may be achieved with nematode-destroying fungi, including nematode-trapping, endoparasitic, egg- and cyst-parasitic fungi, and toxin-producing fungi whose action is concentrated in the faecal environment and directed against free-living stages. More than 200 candidate fungus species have been reported (Tunlid, 2007): to date, *Duddingtonia flagrans* is the most widely studied (Assis et al., 2015). *D. flagrans* develop specific mycelial structures, so-called trapping devices, after induction by nematode or bacterial products (Arias et al., 2013; Li et al., 2016). These devices trap nematode larvae, typically L_3, followed by penetration of the cuticle and complete destruction of the larvae (da Cruz, Araujo, Molento, Damatta, & de Paula Santos, 2011). Resting spores (chlamydospores) of *D. flagrans* may pass through the digestive tract of livestock and develop mycelia in the faeces alongside GI nematode larvae, which are then trapped and killed (Fontenot, Miller, Pena, Larsen, & Gillespie, 2003).
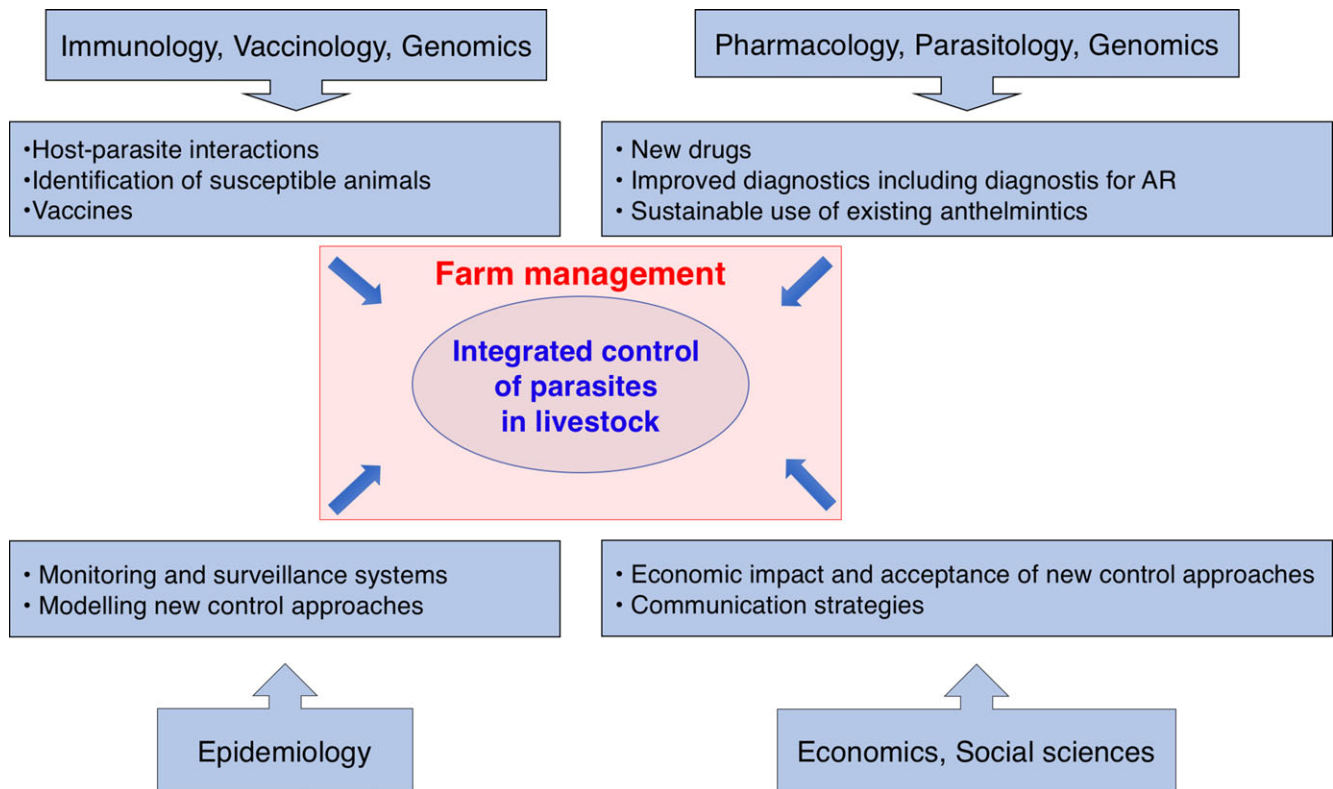
FIGURE 1 Key focus areas for future research to advance the control of gastrointestinal nematodes in ruminants and pigs

Reductions in larval yield from in vitro cultured faeces as a result from fungal infection range from 54% to 100% compared with controls (Paraud, Pors, Chicard, & Chartier, 2006; Rocha, Araujo, & Amarante, 2007), and similar promising effects have been obtained in studies with grazing sheep, goats and cattle (Chandrawathani et al., 2004; Terrill, Miller, Burke, Mosjidis, & Kaplan, 2012). However, efficacy is only reached when the chlamydospores are frequently fed (every second or third day). In addition, in cattle, efficacy was impaired when high FEC coincided with dung pat degradation due to rainfall (Dimander, Höglund, Uggla, Spörndly, & Waller, 2003). Metabolites and other natural products from fungi may also serve as biological control agents, and, for example, substances of the oligosporon type have shown some in vitro activity against *H. contortus* (Degenkolb & Vilcinskas, 2016). However, several of these compounds are unstable in pure form, and at present, they have not been explored for use in livestock.

The major gap regarding the use nematophagous fungi as an effective control method is the lack of a method for regular and frequent delivery of spores. Techniques need to be improved, such as incorporation into feed pellets, incorporation into feed blocks or slow-release boli and combination with inducers to promote the production of chlamydospores by the fungi (Assis et al., 2015; Federica, Alberto, Emilia, Carina, & Alfredo, 2013). Research is hampered by the lack of a commercial source of the spores, which is required to produce sufficient *D. flagrans* for animal trials or other applications (Arias et al., 2013; Terrill et al., 2012). Likewise, a regulatory framework for approval for this type of product, including Good

Manufacturing Practices accreditation and environmental impact assessment, needs to be developed. Research is required to assess whether multi year use of *D. flagrans* (or other relevant species) can progressively reduce GI nematode larval numbers on pasture, reducing infection rates, and improving animal performance (Terrill et al., 2012). Fungal control methods should also be trialled in combination with traditional worm management practices, such as evasive grazing strategies (Hoste & Torres-Acosta, 2011). Finally, more efficient (molecular) screening methods to identify nematophagous fungi expressing a higher trapping rate could be important to this field (Andersson et al., 2014).

8 | CONCLUSION

Key focus areas for future research to advance the control of GI nematodes in ruminants and pigs are graphically presented in Figure 1. A general road map to develop and implement improved control approaches for increased farm profitability, animal health and food security is given in Figure 2. Because of their global distribution and high prevalence, GI nematodes are among the pathogens with the greatest impact on animal productivity. Their impact needs to be further understood and mitigated to meet the future challenge of food security and to enhance production with a reduced environmental impact. The efficacious control of GI nematodes is threatened by a continuing spread of AR, and there is an urgent need for a better understanding of the mechanisms and factors associated
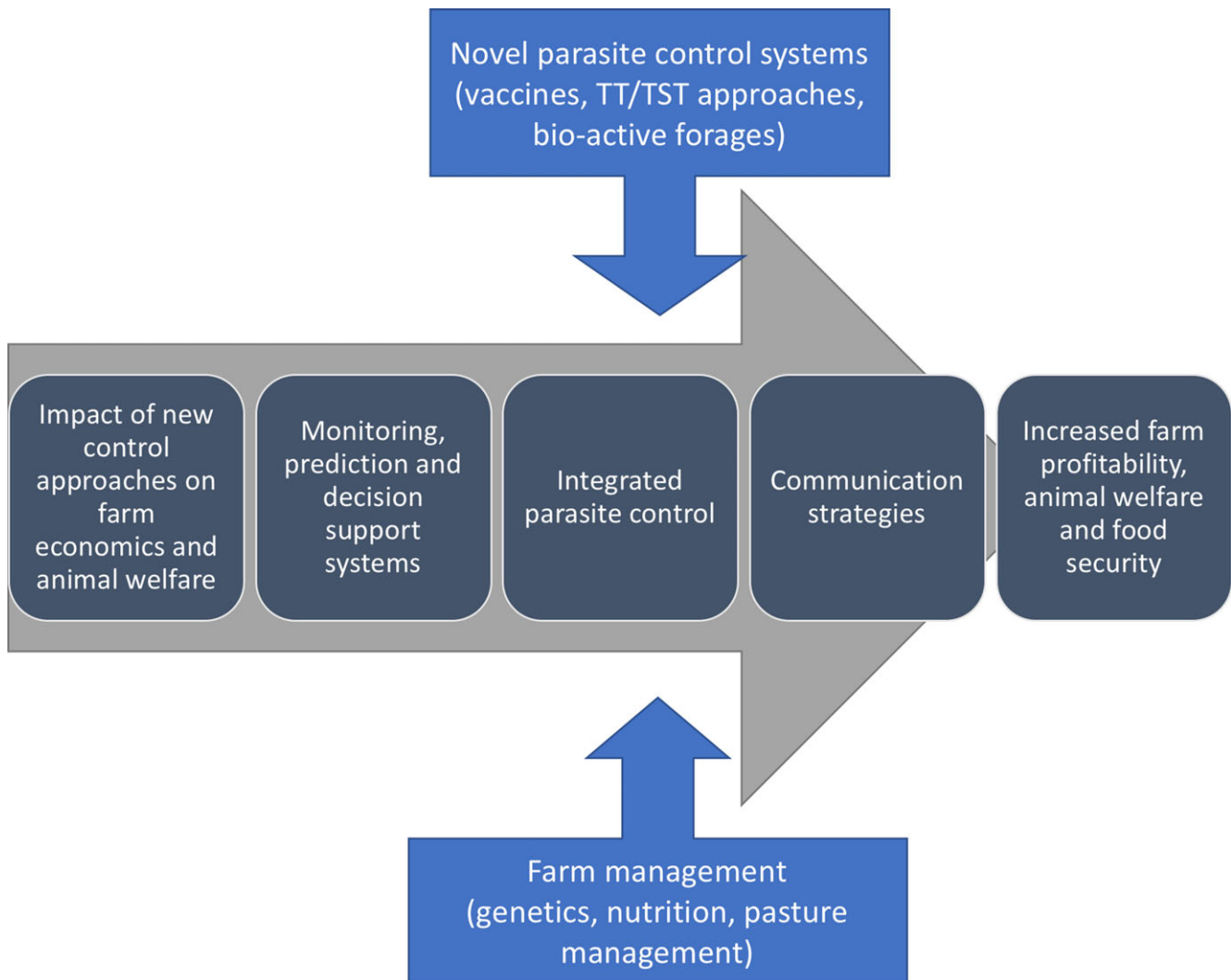
FIGURE 2 A general road map to develop and implement improved control approaches for gastrointestinal nematodes in livestock

with the development of AR. Also, new methods are required allowing to use current and future anthelmintics as selectively and sustainably as possible without reducing productivity or ideally by enhancing productivity. In addition, alternative control approaches are needed, with vaccination and bioactive forages being considered the most desirable solutions in resource-rich and resource-limited circumstances, respectively. Over the last decade, considerable progress has been made and several new diagnostic tests and platforms, targeted selective treatment approaches as well as the first commercially available vaccine against a GI nematode species (i.e., *H. contortus*) have come to reality. However, further work is needed to improve our diagnostic capabilities (i.e., more specific, cheaper, multiplex, pen-side, information on resistance status). Advancing the areas of vaccine and drug development, diagnostics and AR requires basic research in the areas of parasite genomics, transcriptomics, proteomics and metabolomics. Finally, we also need to better understand farmers' and consumers' motivations and beliefs around acceptance of new technologies, to develop vaccines against other/

more GI nematode species and other novel approaches through to a commercial reality.

The solution for sustainable nematode control is not only dependent on more research and development. Regulation is needed to create an environment supportive of innovation in this area. In this assessment, the process to promote "best practice" use of existing products, the development of new multi-active products, as well as to develop guidelines for the requirements for vaccines against GI nematodes needs to be accelerated.

his support and comments on the manuscript regarding the section on nematode-destroying fungi. This publication was only possible thanks to the network of researchers formed under the EU funded projects CAPARA, PARASOL and GLOWORM. This article is based upon work from COST Action COMBAR CA16230, supported by COST (European Cooperation in Science and Technology).

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