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Review

A 'green' toolbox: non-chemotherapeutic approaches for gastrointestinal nematode control in ruminants

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Grazing ruminants are commonly infected with gastrointestinal nematodes, and their negative impacts on animal health, welfare and production are intensified by increasing anthelmintic drug resistance. The goal of reducing anthelmintic use while preserving effective parasite control has motivated research on non-chemotherapeutic interventions, including those relevant to 'green'/organic ruminant production systems. However, 'green' control strategies are at varying levels of development, and an updated overview of the fragmented evidence is timely before they can be integrated with current parasite management. Here, we highlight recent scientific progress of selected non-chemotherapeutic tools for gastrointestinal nematode control in ruminants, existing knowledge gaps, and how novel research approaches and new technologies are contributing to their testing, further development and on-farm implementation, while advancing our understanding of host–parasite–environment interactions.

Non-chemotherapeutic nematode control in ruminants: where do we stand?

Grazing ruminant livestock worldwide are commonly infected with gastrointestinal nematodes (GIN), leading to negative impacts on animal health, welfare and productivity that are intensified by increasing anthelmintic drug resistance [1]. The risk of environmental toxicity from antiparasitic compounds excreted by treated animals [2] further discourages intensive anthelmintic use. GIN are particularly relevant in **'green' ruminant production systems** (see Glossary), which rely heavily on outdoor grazing, leading to higher parasite exposure and infection risks that can be further exacerbated by restrictions on anthelmintic usage [3]. 'Green' farms are increasing worldwide, with certified organic agriculture now practised in 188 countries and an increase of 124% in the global organic farmland area between 2012 and 2022, reaching 96.4 million hectares; of this, two-thirds are permanent grasslands, mainly in Oceania, Latin America and Europe [4]. Ruminant livestock are crucial in 'green' production systems as sources of nutrient-dense foods, and provide organic fertilisers and ecosystem services [5]. Numbers of organic cattle, sheep and goats have increased 33–80% in the last decade in the EU alone[i], while the overall European ruminant population has declined[ii]. This trend will continue following the EU's Farm to Fork and Biodiversity strategies which aim to increase the European organic farmland area from 9.1% in 2020 to 25% by 2030[iii]. However, the lower productivity of organic farms compared with conventional systems is a limitation to the expansion of organic livestock [6] and this yield gap can be exacerbated by poor control of diseases such as nematode parasitism.

The goal of reducing reliance on anthelmintic drugs while preserving effective parasite control has motivated research on non-chemotherapeutic interventions, including those relevant to 'green'

Highlights

Research in a 'green' toolbox for gastrointestinal nematode control has resulted in significant progress for parasite management in ruminants, including host genetic improvement, vaccines, dietary modulation of host immunity and infections, grazing management and biological control.

Novel research approaches and technologies have been explored to address mechanistic and applied challenges.

'Green' strategies may achieve modest initial efficacy independently, but integration of several tools could reduce nematode burdens in the host and lead to cumulative effects on parasite populations.

Combination of 'green' tools requires better understanding of their interactions through empirical and modelling studies.

Future development of 'green' interventions may enhance their efficacies and on-farm implementation, while advancing our understanding of host–parasite–environment interactions.

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ruminants as highlighted in 2004 [7]. Since then, there has been substantial progress in this direction, with notable commercial developments including an anti-*Haemonchus contortus* vaccine [8], *Duddingtonia flagrans*-based (fungal) products for the biological control of GIN [9] and breeding programmes for host resistance towards nematodes [10]. Nevertheless, such advances are restricted to certain helminth/livestock species and countries, and recent surveys have reported limited uptake of alternatives to antiparasitic drugs even by organic farmers [11,12]. Research on non-chemotherapeutic approaches is at varying levels of development, and an updated overview of the fragmented evidence is warranted before they can be integrated into parasite management programmes. Dramatic increases in drug-resistant nematode populations in small ruminants and, more recently, in cattle over the last two decades [1,12,13] has further stimulated investigation of alternatives to anthelmintics. Therefore, it is timely to review the options in a **'green' toolbox** for nematode control in ruminant livestock. Here, we highlight recent advances in selected non-chemotherapeutic tools for GIN control in ruminants, focusing on defining key concepts, reviewing evidence of their *in vivo* effects, identifying knowledge gaps and trade-offs, and discussing how novel research approaches and new technologies can contribute to their testing, further development and on-farm implementation. In addition, we explore the role that **mechanistic/mathematical models** generating **simulations** of GIN populations and host–parasite–environment interactions [14] can play in the evaluation of 'green' tools, including the understanding of their cumulative anthelmintic effects across the nematodes' life cycle.

Ruminant GIN populations can be divided into: (i) parasitic stages in the host, responsible for the contamination of pastures with eggs, and (ii) free-living stages on pasture, responsible for host infection. Non-chemotherapeutic strategies can break the parasite life cycle by targeting these subpopulations (Figure 1, Key figure).

'Green' tools targeting nematode parasitic stages in the host

Host genetic improvement

Host organisms can mitigate the effects of parasitic nematodes through resistance (limiting parasite burden) or tolerance (maintaining health or productivity despite parasitism) [15]: together, these combine to produce resilience to infection (Box 1). Breeding ruminants to enhance resilience to GIN could be cost-effective, long-lasting and environmentally sustainable, and is consistent with organic farming principles. However, progress on host genetics has modified several expectations held 20 years ago [7], such as moving from the hope of identifying single loci underpinning disease resistance, towards the understanding that host defence against GIN is a polygenic trait underpinned by numerous genomic regions (i.e. many loci of small effect) and molecular mechanisms [16]. Recent advances in genomic tools are transforming livestock breeding and can be harnessed to accelerate trait selection to mitigate nematode parasitism.

Which animal trait(s) can be measured and should be selected to mitigate the impact of GIN? Host resistance can be measured using nematode faecal egg count (FEC) and nematode-specific antibody responses, but tolerance is harder to measure, since data on both parasite burden and productivity are needed [15]. Resilience, as the combined effect of resistance and tolerance, can be measured as productivity under exposure to parasites [17], or need for anthelmintic intervention. Selecting for different traits may have different consequences for individual hosts and GIN epidemiology. For example, reduced FEC in resistant animals may lower pasture contamination with infective third-stage larvae (L3) overtime. Mathematical models of weather-dependent GIN dynamics on pasture combined with empirical data, predicted that lambs of resistant ewes would be exposed to reduced larval challenge in their first grazing season [18]. Additional simulations suggested that host resistance could also mitigate predicted future impacts of climate change on infection pressure [18]. Importantly, different trade-offs can be expected between

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Key figure

Overview of the non-chemotherapeutic tools for gastrointestinal nematode (GIN) control in ruminants reviewed in this article

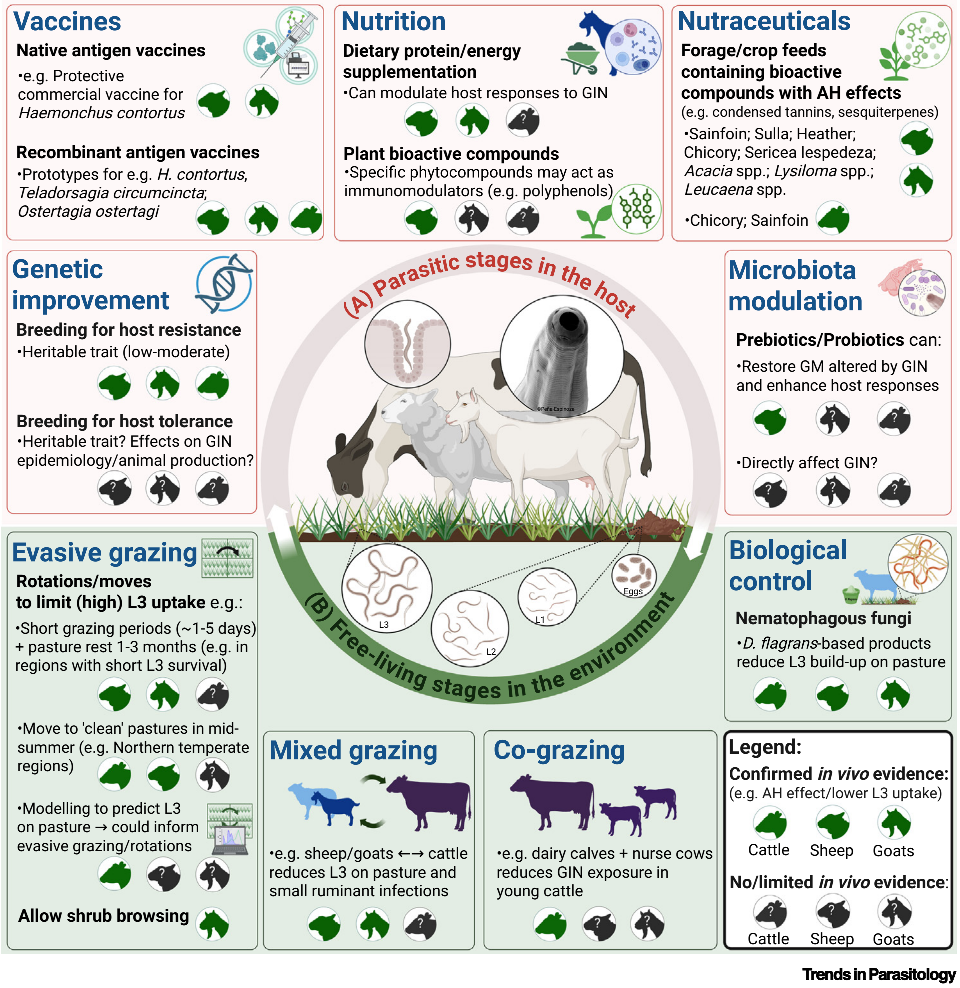

Figure 1. The major ruminant GIN species (e.g. *Haemonchus contortus*, *Ostertagia ostertagi*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*) are pasture-borne with a direct life cycle, and the nematode population can be divided into: (A) parasitic stages in the host, responsible for the contamination of pastures with eggs, and (B) free-living stages in the environment, responsible for infection of the host. Non-chemotherapeutic approaches can break the GIN life cycle by targeting these different subpopulations. For each 'green' tool, it is indicated whether confirmed *in vivo* evidence exists or applicability in live animals has been tested in cattle, sheep and goats. When no ruminant species is depicted, the specific intervention is not applicable. Abbreviations: AH, anthelmintic; Chicory, *Cichorium intybus*; *D. flagrans*, *Duddingtonia flagrans*; GM, gut microbiota; Heather, *Calluna vulgaris*; L3, infective third-stage larvae; Sainfoin, *Onobrychis viciifolia*; Sericea lespedeza, *Lespedeza cuneata*; Sulla, *Hedysarum coronarium*. Created in BioRender. Peña-Espinoza, M. (2025) https://BioRender.com/y318pr2.

host resistance/tolerance to GIN, animal productivity and pasture contamination. For example, immune responses are costly for individual hosts and selection of resistant animals can lead to lower productivity [19]. *H. contortus*-resistant sheep have shown variable trade-offs between host immunity and resource reallocation, with energy allocated to immunity being three times

Glossary

Bioactive compounds (BC): also known as plant secondary metabolites, are a diverse array of phytochemicals synthesised by certain plants related with defence against herbivory, e.g. condensed tannins (proanthocyanidins) and sesquiterpene lactones. Ongoing research has confirmed the bioactivities of these phytochemicals in various biological systems, including antiparasitic, antimicrobial, anticancer, antioxidant and immunomodulatory effects.

'Clean' pastures: pastures with no or very low contamination with GIN eggs/larvae (e.g. new ley or ungrazed pastures used for hay/silage cut; pastures not grazed by ruminants for ≥12 months; undersown crop from the previous season).

Digital shadows: in the context of this review, a digital representation (mathematical model) of a farm system and the biological processes underlying GIN epidemiology, which can use farm data (e.g. longitudinal FEC, grazing and weather data) to drive simulations. These simulations can then be used to inform decision-making and manually perform a farm management action, such as the application of particular control methods (e.g. pasture rotation; targeted feeding with nutraceuticals).

Digital twins: similar to a digital shadow, but the resulting model simulations are used in real time to automatically change a farm management action (e.g. changing ratio of feed components in an automated feeding system).

Genomic prediction: a method for refining the estimate of how closely related animals are to each other based on shared genetic sequence data rather than average relatedness from pedigree structure alone. It enables more accurate estimation of genetic parameters such as trait heritability and genetic correlations, and the prediction of breeding values for a given trait without needing to phenotype the animal.

'Green' ruminant production systems: in the context of this review, farming systems pursuing certified organic farming, agro-ecology, regenerative agriculture and other low-input agricultural practices that rely heavily on grazing and outdoor rearing, while bound to a restricted use of veterinary drugs (e.g. anthelmintics).

greater in genetically resistant versus susceptible sheep lines [20], although this higher immune energy costs in resistant animals is predicted to be limited and transitory [21]. Genetic correlations between GIN resistance and productivity in sheep can vary from strongly negative to strongly positive based on the resistance trait measured, with overall favourable correlations and higher productivity when selecting resistant animals based on FEC (and FAMACHA© score) than when selecting for immune-related traits [10]. In contrast, tolerance to GIN is unlikely to involve immunity costs in the host and thus productivity may not be affected [15], but tolerant animals will increase pasture contamination with nematode eggs compared to resistant hosts [22]. The consequences of host genetic improvement will therefore depend on how resilience is underpinned by resistance and tolerance at the individual and herd levels [23] (Box 1). Currently, considerably more is known regarding genetics and breeding for resistance than resilience and tolerance in ruminants.

Breeding to enhance a trait requires it to be heritable. A recent meta-analysis of 121 trials found that sheep resistance to GIN (based on FEC, FAMACHA© score and immune responses) had a global heritability estimate of 0.25 across breeds, ages and locations [10]. Single studies of genetic resistance in Asian goats [24] and African cattle [25] have reported similar (low to moderate) heritability. Genotype-by-environment interactions also occur whereby genetic parameters vary with geographic location and infection pressure [26], parasite species [27] and breed [28]. Resilience is heritable when measured as age at first drench or weight gain from weaning [17]; Romney sheep bred for resilience weigh more, have lower FEC and require their first deworming later than control animals [29]. In contrast, tolerance to GIN has been poorly studied, probably because is difficult to measure and concerns about increasing pasture contamination [22], although breeding for tolerance has been advocated [30] and cattle breeds differ in tolerance to other helminth infections, e.g. liver fluke [31]. The mechanisms and consequences of host tolerance to GIN in ruminants remain unknown, and its potential as a breeding trait demands further research.

In livestock breeding, phenotype, population pedigree and trait heritability are combined to calculate estimated breeding values (EBVs); individual animals with the best EBVs for a trait are selected for breeding [32]. Genetic improvement by EBVs can be successful but is slow if the trait is difficult to phenotype. Genomic technologies now enable the selection of animals without phenotypic information if they have a genotype favourably associated with the desired trait. High-density marker chips enable genotyping of animals at thousands of single-nucleotide polymorphisms (SNPs) [32], and genome-wide association studies have analysed associations between SNPs and phenotype, identifying hundreds of quantitative trait loci (QTLs) associated with resistance to GIN [16]. Downstream analyses show many of these are related to immune function and gastrointestinal physiology [16]. The declining costs of whole-genome sequencing (WGS) and development of imputation methods are further improving power to detect QTLs [33]. While many QTLs associated with resistance to GIN have been identified in sheep [16], results are inconsistent and typically explain only a few percent of the genetic variation [34]. New breeding approaches based on **genomic prediction** using SNP data can now calculate genomic breeding values (GEBVs) that provide more accurate parameters for selection than classical EBVs [35]. Still, a large reference population with genotype and phenotype information, and variation across breeds, is required for genomic selection [36], meaning that a reference population from one breed will not yield accurate GEBVs for another [32]. Nevertheless, genomic prediction and accurate estimation of GEBVs is being advanced by progress in sequencing technologies and statistical approaches, including machine learning modelling [37], which combined with improved assisted reproductive technologies could accelerate the rate of genetic selection for animal resistance to pathogens [38].

While certified organic farming is practised following international principles from IFOAM – Organics International [4] and national/supranational regulations (e.g. EU Organic Production Regulation (EU) 2018/848), 'green' farming practices such as agro-ecology and other sustainable/environmentally friendly approaches are much broader and are not limited to specific regions/countries, farming systems or certifications.

'Green' toolbox: non-chemotherapeutic tools developed to prevent and control GIN that can be implemented in 'green'/organic and conventional ruminant systems to reduce reliance on anthelmintic drugs.

Mechanistic/mathematical model: a simplified, mathematical representation of a system, representing the biological processes underlying GIN epidemiology and GIN–host–environment interactions.

Nutraceuticals: in livestock farming, a forage/crop feed combining high nutritional value with beneficial effects on animal health (e.g. direct antiparasitic activity and/or immunostimulatory effects) derived from its content of bioactive compounds.

Simulation: implementation of a mechanistic model to generate a predicted outcome (e.g. FEC profile) for a specific scenario/set of conditions in a physical system.

Box 1. Strategies of host defence against nematode infection

There are two broad strategies through which host organisms can mitigate the impact of nematode parasites on their health and fitness. The first strategy is to actively fight parasites, by reducing their rates of establishment, fecundity and development, or expelling them; this is known as RESISTANCE, which in the case of GIN infections of sheep relies on a very well-characterised immune response [110]. One way of measuring resistance in GIN-infected animals is with a FEC; animals with lower FEC are assumed to be more resistant. For example, when comparing two groups of individuals (e.g. two different breeds, or genetic lines) exposed to the same level of GIN, the group with the lower FEC are presumed to be more resistant (Figure IA; red group). Note that here animal performance has not been shown: resistance says nothing about an animal's productivity. The second strategy is not to confront the infectious agent, but rather its effects, by preventing or repairing damage or neutralising toxins [111,112]; this is known as TOLERANCE and can be measured as the slope of a desirable trait (e.g. growth rate or milk yield) on infection burden, such that more tolerant hosts show a lower decline in performance at a slower rate as infection burden increases [15]. For example, when comparing two groups, those able to maintain a more consistent growth rate/milk yield in the face of increasing nematode burden (e.g. FEC) are more tolerant (Figure IB; blue group). Despite the different strategies towards similar parasite exposure, both groups have equal productivity overall (Figure IC) and hence equal RESILIENCE, which is the product of resistance and tolerance. The two groups are equally resilient, but the red group derives this resilience from being more resistant, while the blue group derives it from being more tolerant. It is important to note that resistance and tolerance are not mutually exclusive, and that most individuals will lie on a continuum of both traits, rather than exhibiting one or the other.

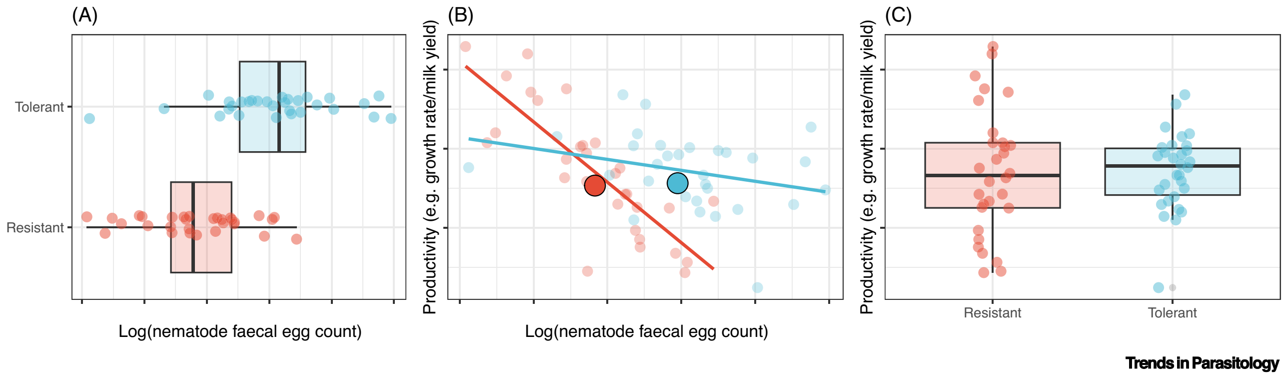

Figure I. Example profiles of host resistance (A), tolerance (B), and resilience (C) in two groups of animals exposed to gastrointestinal nematodes. Small translucent points show individual measures of nematode faecal egg count (FEC; panel A), FEC and productivity (panel B), or productivity (panel C); boxes (panels A and C) show median and interquartile range; large solid points (panel B) show means of FEC and productivity; lines (panel B) show the slope of the relationship between productivity and FEC. The data has been generated by the authors to allow clarity of the concepts.

Vaccines

A landmark of the last 20 years has been the commercial release of an anti-*H. contortus* vaccine (Barbervax®/Wirevax®) containing native, gut-derived antigens (H-gal-GP and H11) [8], which is available in Australia, the UK and South Africa for conventional and organic farms[iv]. This vaccine induces strong protective immunity in small ruminants to *H. contortus*, stimulating high antigen-specific circulating antibodies and resulting in significantly lower FEC, worm burdens, clinical signs and pasture contamination with L3 in temperate and tropical environments [8,39]. Barbervax® requires priming vaccinations of young animals with repeated boosters to maintain protection, and booster vaccination of pregnant ewes [40] and of pregnant goats [41] can enhance protection after lambing/kidding and during lactation. However, the fact that Barbervax® remains the sole commercial GIN vaccine for ruminants highlights challenges in developing vaccines based on native parasite antigens, which require isolation of adult nematode proteins harvested from infected animals, limiting upscaling of production and posing risks of carrying adventitious agents.

Although several protective native GIN antigens have been identified, no protective recombinant versions have been generated for commercial development to date. There is experimental evidence that protective recombinant vaccines can be developed, for example for *Teladorsagia circumcincta* [42]. New insights into host immunity and parasite genomics may enable the development of recombinant subunit-based vaccines that are affordable, safe, scalable and target multiple GIN species. Key requirements for success include: (i) identifying protective antigen targets; (ii) producing recombinant versions which retain protective epitopes; and (iii) inducing appropriate effector and memory immune responses at the site of infection.

Traditional strategies to identify protective antigens have relied on immunising animals with native parasite extracts and/or screening of immunoreactive nematode proteins [42] using proteomics and mapping peptides to reference genomes; however, the lack of well-assembled GIN genomes may have resulted in selection of antigens with high functional redundancy. This situation is changing with high-quality genome assemblies now available, for example, for *H. contortus* [43] and *T. circumcincta* [44]. Furthermore, the recent ability to perform WGS on single male worms allows the generation of well annotated chromosomal-level genome assemblies in a matter of weeks[v,vi], potentially improving the identification of candidate vaccine antigens through selection of conserved/less variable targets. Another approach to vaccine antigen selection is to identify proteins crucial for parasite establishment, reproduction and/or survival within the host. This requires detailed molecular-level dissection of host–parasite interactions, and this is now possible following advances in organoid cultures enabling the *in vitro* study of key processes involved in parasite infections, such as invasion of gastric glands by infective L3 [45,46].

Once promising antigen targets have been identified, synthesising recombinant versions that retain protective epitopes remains a challenge, as illustrated by the lack of protection conferred by recombinant vaccine candidates for *H. contortus* (H-gal-GP and H11) and *Ostertagia ostertagi* (Oo-ASP-1). For H-gal-GP, this appears to be due to the lack of key conformational epitopes [8], whereas for H11 and Oo-ASP-1 N-glycosylation of the native protein appears to be crucial for protection [47,48]. Encouragingly, recent advances in recombinant protein expression have allowed glyco-engineered versions of Oo-ASP-1 in *Nicotiana benthamiana* plants [48] and of H11 and GA1 from *H. contortus* in insect cells [49], which mimic the N-glycan composition of the native protein and can confer protection. Such approaches should be applicable for other GIN vaccine candidates where glycan residues are important for protection.

Finally, for optimal protection, the effector host immune response must act at the site of nematode infection, the gastrointestinal mucosa. The optimal method would be direct vaccine administration onto the mucosa, but this is challenging in ruminants [50]. Several mucosal delivery systems have shown promise in GIN vaccines including mucoadhesive gels, mucosal adjuvants, live attenuated bacterial vectors and microparticles [51], although these systems generally induce poor levels of protection. To date, the most successful ruminant GIN vaccines have been delivered subcutaneously or intramuscularly, together with conventional adjuvants [51]. Yet, systemic vaccines may not efficiently induce immune memory at the mucosal site [52], meaning that on-site natural boosting may be less effective, with implications for duration of immunity. Promisingly, there is some evidence that systemic GIN vaccination can also prime gastrointestinal sites [53].

Modelling can drive further refinements in vaccine design by accounting for non-linear responses and cumulative epidemiological benefits of FEC reductions over time, helping to estimate the minimum vaccine efficacy and coverage required for vaccine candidates in the field. Early GIN models predicted that vaccines with 60–80% efficacy (in 80% of vaccinated animals) could result in flock protection and reduce animal deaths [51,54], whereas for Barbervax® a minimum vaccine efficacy of >65% was anticipated as required to induce an epidemiological effect and adequately control *H. contortus* [55]. More recent models of GIN population dynamics (e.g., [56,57]) are promising tools that can advance the technology readiness level of vaccines, e.g. during the proof-of-concept and preclinical development phases. Models can also be used to make inference about 'unobserved spaces': parameters, interactions, and mechanistic drivers that are impossible to fully assess empirically or are yet to be evaluated. For example, multivariate statistical models have generated insights into interacting drivers of vaccine protection [58]. This, aligned with improved recombinant protein technologies will increase the likelihood of achieving protective subunit GIN vaccines.

Nutrition and host responses

Plane of nutrition is critical in sustaining host responses to parasitism and its consequences; malnourished animals and those under high nutritional demand are more susceptible to infection [59]. This framework has guided practical recommendations of improved nutrition to enhance host resistance and restore nutrient loss in parasitised ruminants for decades [7]. Research progress in the last 20 years has built on these principles, and also expanded our understanding of the role of specific dietary components on host immunity during helminth infection.

Dietary protein and energy are well-studied examples of nutritional modulation of host immunity towards GIN [60,61]. Sheep with suboptimal dietary protein intake are more susceptible to GIN, and provision of additional protein rapidly restores resistance and boosts the production of anti-helminth antibodies and mast cells [62]. Whilst long-term supplementary protein feeding is prohibitively expensive, short-term targeted supplementation during times of high nutritional stress (e.g. in peri-parturient animals) [60], or in combination with vaccination [39], have demonstrated beneficial effects on performance and reduces the need for anthelmintic treatments. In goats, studies mainly from tropical regions suggest that supplementary feeding with both dietary protein and energy is needed to boost host responses towards GIN, as adequate levels of rumen-fermentable energy can enhance protein metabolism, particularly in animals with low energy/body fat reserves [61,63]. However, it may be difficult to disentangle the specific contributions of dietary protein and energy on host responses to GIN, as protein–energy interactions occur in several metabolic pathways and physiological functions [61]. In cattle, much less is known about the effects of supplementary feeding on host responses to GIN, and the limited experimental evidence dates back >20 years [64].

A notable advance in the last two decades is the recognition that not only can overall improved nutrition enhance animal responses to GIN, but specific dietary components such as plant **bioactive compounds (BC)** or the type and/or amount of dietary fibre may also act as immunomodulators, independently of improved nutrition per se [65]. For example, bioactive polyphenols such as tannins can directly bind to and activate sheep lymphocytes [66], and sheep grazing tannin-rich pastures have higher levels of immune cells concomitant with a transient reduction in FEC [67]. Whether specific immune responses can be promoted to enhance anti-GIN immunity will be an increasingly pertinent avenue of research. In addition, the modulation of GIN-gut microbiota interactions in the host is being increasingly explored and may lead to novel dietary interventions to limit nematode parasitism and its consequences (Box 2).

Nutraceuticals

Research on bioactive forage/crop feeds as **nutraceuticals** for the dietary modulation of nematode infections has greatly expanded over the last decades, with confirmed *in vivo* effects of certain temperate and tropical plants against GIN in small ruminants, and more recently, in cattle [60,68,69]. It is well-recognised that the anthelmintic activities of nutraceutical plants derive from their content of dietary BC [7], which can directly target parasitic stages [60,69], and the recent integration of parasitological studies with in-depth metabolite profiling is revealing the role of specific molecules/molecular conformations on their antiparasitic effects.

Box 2. Modulation of nematode–host gut microbiota interactions: A novel 'green' tool?

The role of host gut microbiota (GM) in health and disease has been a major revolution in biomedical sciences, and the importance of GM composition on animal health and productivity is increasingly recognised [113]. Manipulation of the GM in livestock, either by dietary interventions that promote the growth of certain bacterial taxa, or by the direct administration of live microorganisms with beneficial properties (i.e., probiotics), has potential to promote animal health and reduce antimicrobial usage. Studies in sheep and goats have suggested that the abomasal and/or faecal GM composition is indeed different in parasitised hosts, compared to their uninfected counterparts [114,115]. How these modified bacterial communities impact the animal, and to what extent they contribute to reduced productivity associated with GIN, is not yet clear. GM-modifying interventions can either correct dysbiosis associated with infection, thus restoring the GM to its 'natural' state, or enhance the ability of the host to remove the infection by boosting immunity or restructuring the GM to a composition that is unfavourable for GIN. Several dietary interventions can attenuate GIN-induced changes in host GM composition. In sheep, feeding the tannin-rich plant *Acacia mearnsii* restored populations of butyrate-producing bacteria that were depleted in the rumen by a mixed *Haemonchus contortus* and *Trichostrongylus colubriformis* infection [116]. Supplementation with yeast probiotics such as *Saccharomyces cerevisiae* or *Saccharomyces boulardii* decreased *H. contortus* FEC, concomitant with the enhancement of immune parameters [117,118]. However, evidence from mono-gastric livestock suggests care is warranted as administration with some probiotic bacteria may increase GIN infection, and inhibit type-2 immune responses that are important for parasite clearance [119]. Furthermore, the practical delivery of feed additives to modulate the GM may be challenging in grazing animals, particularly in extensive systems. Potentially, certain pasture compositions (e.g. specific forages) containing a phytochemical profile known to selectively boost specific GM population(s) that protect against GIN may be utilised for grazing. Alternatively, on-farm administration of probiotics may be more practical in dairy animals with daily milking or in more intensive farms with controlled dietary rations. In extensively grazed ruminants, 'long-acting' probiotics (i.e. those which may colonize the host gut) could feasibly be administered at the same time as animals are mustered for drenching or other routine management procedures. Similarly to other 'green' tools for GIN control, probiotics and GM modulation may not be a one-size-fits-all solution and will have to be specifically tailored to certain production systems.

Several classes of plant BC have been tested for anthelmintic activity *in vitro*, but most *in vivo* trials in the last 20 years have focused on nutraceutical plants rich in either condensed tannins (polymeric polyphenols; CT) or sesquiterpene lactones (terpenes; SL). In temperate areas, feeding ruminants with CT-rich plants such as sainfoin (*Onobrychis viciifolia),* sulla (*Hedysarum coronarium*), heather (*Calluna vulgaris*) or sericea lespedeza (*Lespedeza cuneata*), or SL-rich forages like chicory (*Cichorium intybus*), can affect parasite development, survival and fecundity, leading to reduce nematode burdens and/or FEC by up to ~60% [60,68,69]. Feeding certain tropical plants, including some *Acacia* species, *Lysiloma latisiliquum* and *Leucaena leucocephala*, have also shown *in vivo* effects against GIN [68,70], although their anthelmintic activities are reportedly lower than temperate forages. Apparently, feeding with nutraceuticals may be selective against different nematode species. For example, cattle fed with sainfoin pellets or forage chicory had a significant reduction of the abomasal *O. ostertagi,* but not the small intestinal *Cooperia oncophora* [71,72], whereas feeding with sainfoin in sheep has led to either reductions of only abomasal worms or only small intestinal nematodes [60]. Given that plant CT or SL can directly induce anthelmintic effects towards different GIN species *in vitro* [60,69] the basis for these *in vivo* variabilities may derive from complex host–parasite–plant interactions, including: (i) the initial BC concentration in the original feed, (ii) changes in dietary BC concentration and composition reaching different gastrointestinal compartments [73], (iii) the interactions between different dietary BC in the host, and (iv) intrinsic differences in GIN species' susceptibilities to BC [60]. Although the development of nematode resistance against plant molecules may be limited by the simultaneous exposure of GIN to various phytocompounds in the feed, even acting synergistically [74], parasite adaptation to BC needs to be considered following the variable susceptibilities of different *H. contortus* isolates to natural polyphenols [75].

One key aspect for the implementation of nutraceuticals as control strategy is to define the dietary levels needed to effectively reduce parasite burdens. Inclusion levels of >50% nutraceuticals in the diet (based on dry matter intake) have been reported to induce anthelmintic effects [72,76], although levels of 20% may still be effective for some plants [77]. Differences in palatability may regulate voluntary intake levels, particularly when the feed is offered ad libitum [68], and a nutraceutical feeding of >7 days may be required to ensure that sufficient BC reach the digestive organs and target parasites [60]. Ultimately, the lower effective intake level for nutraceuticals is not clear and will depend on the concentration and type/molecular profile of the BC in the plant, which in turn will vary depending on plant genetics, cultivar/accessions and local growth conditions [60,78]. High intake of certain bioactive plants can result in a trade-off with reduced feed intake and animal performance [77], although this is not the case for all nutraceuticals [69], and it may be influenced by the plant source/type of the BC [60]. Furthermore, dietary BC such as CT are being increasingly explored as tools to reduce methane emissions in ruminants [79], and further research needs to determine ideal inclusion rates to achieve health/environmental goals without affecting animal performance.

Despite growing interest on BC-rich grasslands with health benefits for livestock [80], cultivating fresh bioactive forages is not possible in all ruminant farms, and it may be difficult to temporarily match plant growth and nutraceutical availability with GIN exposure during the grazing season. Agro-industrial by-products as novel sources of plant BC could overcome these challenges and ensure the availability of nutraceutical products when infection risk is high [78,81], which may offer more practical alternatives than feeding fresh forages. Finally, a better mechanistic understanding of the main antiparasitic BC could improve and/or standardise nutraceutical efficacy, and new knowledge has been gained by combining parasitology, phytochemistry and metabolomic studies of model plants. For CT, plant material with a higher proportion of subcomponents prodelphinidins than procyanidins, and with CT of high molecular weights, show higher anthelmintic activity [82,83]. In SL-rich plants, 8-deoxylactucin has been identified as the main nematocidal SL in chicory, but with synergistic activity in combination with other molecules in the plant [74]. Thus, by identifying the antiparasitic roles of individual and/or combined BC, these specific molecules could be used as markers of plant material with nutraceutical potential.

'Green' tools targeting nematode free-living stages on pasture

Grazing management

A straightforward approach to limiting the uptake of infective larvae from pastures would be to identify paddocks with low nematode contamination and use these areas for grazing. However, detection and quantification of GIN L3 in the environment is laborious and mostly limited to research projects. In practice, grazing management strategies for nematode control can be: (i) preventative (e.g. turn-out of susceptible stock to **'clean' pastures**), (ii) evasive (e.g. moving animals to new pastures or allowing shrub browsing to limit L3 uptake), or (iii) diluting (e.g. dilution of L3 uptake by lowering effective stocking rates of susceptible hosts) [64]. Although these concepts have been known and used for decades [7], progress in the last 20 years has highlighted the limitations and potentials of grazing strategies for GIN management in different regions.

In evasive strategies, e.g. rotational grazing, the survival rate of infective larvae determines the optimal rest intervals of paddocks, which is expected to be shorter in tropical regions compared with temperate areas [84]. This has validated rotational systems for small ruminants involving short grazing periods of a few days and a return to the grazed paddocks in about one month in areas with assumed short survival of free-living GIN stages [7]. Recent evidence of *H. contortus* L3 surviving for >180 days in subtropical pastures in Brazil [85] challenges this assumption and suggests that rest interval periods of several months may indeed be needed to achieve truly 'clean' pastures also in warm climates. Long-term trials in Australia [86] and Spain [87] exploring intensive rotation of sheep on small 'cells' grazed for 1–5 days with mean rest intervals of 45–100 days have confirmed lower FEC and/or need of deworming, including a shift in the prevalent parasites with reduced *H. contortus* levels over time [86,87]. Still, rapid rotational grazing with long resting intervals may be unpractical (and unprofitable) in farms with limited paddocks. The vertical distribution of infective larvae on pasture can also affect the applicability of specific grazing strategies: whereas in temperate areas most infective larvae are found in the lower part of the sward, in tropical/subtropical pastures *H. contortus* L3 can be detected at various pasture heights [88], indicating varying potential to reduce larval uptake by controlling pasture height between different regions. Moreover, vertical migration and survival of GIN L3 in both temperate and tropical pastures varies with forage species [69,89]. In Northern temperate climates with winter stabling, strategic moves/change of cattle to 'clean' pastures (e.g. at mid-summer) without anthelmintic treatment can reduce high L3 uptake later in the grazing season [64], with similar findings in sheep [90]. Nevertheless, this strategy may not be applicable in other regions and will depend on the GIN species present, length of the grazing season and weather conditions, and care should be taken when move to 'clean' pasture is combined with deworming, as it may rapidly select for anthelmintic resistance due to the limited refugia of drug-susceptible nematode populations.

The survival of infective larvae and their build-up on pasture can be affected by weather, pasture availability and stocking rate, requiring continuous adaptation of evasive grazing schemes to avoid high infection levels. The complex interplay between free-living GIN stages on pasture with abiotic factors and farm management is difficult to forecast, but novel modelling approaches are being implemented to predict pasture contamination that can support grazing management decisions [56,91]. A model that tracks the development, survival and behaviour of GIN infections in cattle on pasture based on farm-specific FEC, weather and rotational grazing data can produce prototype colour-coded 'contamination maps' of specific paddocks within a farm [91]. This approach could enable real-time L3 mapping on pasture using relatively simple simulation models of the nematode free-living stages and regular FEC monitoring, allowing to compare pasture contamination under competing grazing scenarios and to inform evasive grazing strategies if predictions are delivered to farmers. Furthermore, understanding of local parasite epidemiology and the effects of climate change on nematode biology on pasture, including on faecal moisture [92], may need to be updated for different regions to optimise and inform in silico modelling efforts towards the design of evasive grazing plans for parasite management [56,91].

Mixed-grazing strategies are based on the sharing and/or alternate use of pastures by hosts with distinct susceptibilities, such as animals of different age and/or species. Co-grazing dairy calves with nurse cows results in low parasite exposure in young animals, influenced in part by a dilution effect of immune cows (ingesting L3 but excreting fewer eggs) and the milk diet of the calves (thus reducing L3 uptake) [93]. Mixed or alternate grazing between small ruminants and cattle in temperate and tropical regions has been explored based on the host-specificity of GIN, resulting in lower L3 on pasture and infections in sheep/goats, but not in cattle [94,95]. For example, young steers sequentially grazed with lambs had similar FEC and liveweight gains but higher serum pepsinogen levels compared with cattle grazing alone, suggesting increased infection pressure from paddocks shared with sheep, including the confirmation of *H. contortus* in both ruminant species [95]. In a tropical environment, mixed grazing of goats and cattle resulted in lower FEC in weaned goat kids [96], but not in adult goats [97], which could be due to different foraging behaviour (and L3 uptake) between age groups.

Biological control

Several organisms have been explored for the biological control of free-living GIN stages in the environment, including nematode-predatory fungi, dung beetles and earthworms [98]. Of these, the use of nematophagous fungi, particularly *D. flagrans*, has been for decades the most successful biological method to mitigate parasitic nematodes in ruminants [7,99]. Animals fed daily with *D. flagrans* resting chlamydospores excrete these almost unchanged in the faeces, where they develop to hyphae that trap and predate nematode larvae. The benefits of *D. flagrans* for reducing GIN L3 build-up on pasture in field trials have been known for >30 years [7], thus studies in the last two decades have focused on the development of commercial formulations, validation under temperate and tropical conditions, and integration with other parasite control tools [77,98,99]. Industrial research resulted in new *D. flagrans*-based products (BioWorma® and Bioverm®) recently marketed in Australia, New Zealand, Brazil and the US for different livestock species, including ruminants [9,98]. These products are commonly offered to animals mixed with feed and require daily administration, which may be more practical on intensive farms with everyday stabling and seasonal grazing, but less so in large herds with year-round pasturing and limited animal handling. Alternative delivery systems such as mixing spores in nutrient blocks, intra-ruminal controlled release devices or combined with cereal grains have been experimentally validated but lack commercial development [99]. In contrast, pelleted *D. flagrans* shows promise as it retains its activity, can be delivered more easily and evenly in a given herd, and spores remain viable for several years [9,99]. Further research needs to confirm the effects of new *D. flagrans* formulations on pasture contamination and animal productivity under different environmental conditions, given the impact of weather/season on its nematode-trapping activity [100]. Encouragingly, the activity of *D. flagrans* seems to not be affected by concomitant feeding with nutraceutical plants [77], thus validating the potential integration of these tools.

Soil fauna such as dung beetles and earthworms can also affect free-living GIN stages by disrupting the faeces and hindering nematode survival and development, by directly ingesting eggs/larvae, and even by secreting nematode-toxic compounds [98,101]. However, the available evidence shows highly variable effects of these organisms on pasture L3 levels [98], and their disruptive activities on faecal pats may even enhance the number of infective larvae that develop and migrate into pasture in areas of high soil fauna abundance [102]. Although practical interventions with dung beetles and earthworms for ruminant parasite control are yet to be developed, their contributions to soil quality and nutrient cycling will continue to encourage farming practices to promote these organisms, and further ecological studies may help to clarify their role as biological control of GIN in different regions.

Non-chemotherapeutic nematode control in ruminants: where could we go from here?

Non-chemotherapeutic alternatives for nematode control are unlikely to achieve the initial efficacy of modern anthelmintics independently, but integration of several strategies may reach acceptable levels of GIN management. The combination of 'green' tools requires a better understanding of their interactions, which may be obtained empirically or by integrating experimental and simulation studies. Computational modelling can play a key role in the development of 'green' tools and their individual and combined evaluation, and while models are useful, their value is greatest when integrating *in vitro*, *in vivo,* and *in silico* methods (Box 3). Further advancements in coming years may also enhance and/or standardise the individual efficacies of each non-chemotherapeutic approach and help identify the most relevant tools for on-farm implementation in different regions, but specific challenges remain.

In host genetic improvement, a better understanding of the mechanisms and consequences of host tolerance to GIN is needed. On-farm, the next decades will likely experience an increase in the use of genomic tools for in-depth characterisation of animals with desirable traits for breeding. The growth of commercial companies offering genotyping and informatic services to livestock breeders, and the expanding availability of genetic parameters for more traits across more breeds (e.g. facilitated by performance recording on farm), will enable more precise breeding decisions and rapid genetic improvement [103]. The advent of CRISPR/Cas gene editing may also help

Box 3. Modelling and its role in the evaluation of 'green' tools for nematode control

'Green' nematode control interventions not only reduce immediate parasite burden but also have downstream effects on GIN populations, potentially reducing overall pasture infectivity and affecting the establishment and survival during reinfection. These cumulative impacts may lead to benefits exceeding initial expectations from even modest reductions in worm burden or FEC. However, empirically evaluating cumulative effects across the GIN life cycle and multiple host production cycles is costly, requires long-term monitoring, and cannot fully capture the uncertainty in these systems (e.g. genotype–environment interactions [27]). In contrast, modelling studies can simulate the effect of single or multiple non-chemotherapeutic interventions over extended periods and wide parameter spaces, as pioneered 30 years ago [54]. Recent computational modelling of parasite metapopulation dynamics can now simulate multiple 'patches' of pasture nematode subpopulations coupled by host movements between pastures, allowing the relative efficacy of different control strategies to be evaluated [56]. These simulations enable exploration across space [91] and time [18], an impossible feat to achieve on the same scale empirically. The outputs can clarify the reduction thresholds in FEC necessary for novel control methods, as well as the anticipated population-level impacts of tools with 'low' *in vivo* efficacy. Employing weather-dependent mechanistic models to investigate how free-living GIN stages develop, survive, and behave in pasture ecosystems can generate useful insights into e.g. grazing management, sward diversity and biological control options, facilitating practical implementation of these tools. '**Digital twins**', a virtual representation of a system (computational model) bolstered by bi-directional flow of data from the physical system using real-time sensors [120], is a particularly promising field, presenting an opportunity to bridge the gap between research and implementation of 'green' tools in agriculture. This includes the use of livestock motion sensors (e.g. accelerometers and computer vision), automated milking sensors, and real-time weather data. Currently, GIN models that have progressed furthest on this trajectory operate as **'digital shadows'**, partially integrated with unidirectional flow of real-time weather data (e.g. SCOPS, 2025[vii]). However, prototype models such as the pasture contamination mapping model described earlier [91], offer a compelling pathway to the development of digital twins in veterinary parasitology, by real-time input of weather data and output of contamination maps for potential integration with farm mapping and electronic identification software and mobile apps.

to accelerate genetic gain by introducing advantageous alleles from one breed into another, without the need for crossbreeding [104], but this may work most effectively for QTLs with larger effects rather than for polygenic traits such as defence against GIN. An alternative strategy could be multiplex gene editing, enabling multiple genomic regions to be targeted for breeding selection [105]. However, genetic engineering is currently not expected to be readily accepted by the 'green' ruminant sector, particularly in certified organic farming. Nonetheless, one of the principles of organic farming is the selection of robust animals adapted to the farm environment [3], including preservation of local/endangered breeds, which may allow the identification of resistant/tolerant individuals (and their genomic backgrounds) among diverse animal genetic resources for breeding.

Despite the challenges for recombinant vaccine development, recent advances in GIN genomics, improved *in vitro* culture systems, antigen discovery, recombinant protein expression and vaccine delivery are expected to translate in novel commercial vaccines in the medium to long term. As mixed GIN infections are the norm, specific vaccines will need to be either combined, or monospecific vaccines used with a good understanding of parasite epidemiology. The interactions between vaccines and other 'green' tools also need to be further characterised, following evidence in sheep that vaccination and improved nutrition can induce a combined effect against *H. contortus* [39], as well as vaccination and host genetic resistance against *T. circumcincta* [106].

An increased understanding of how dietary components interact with the host immune system should yield targeted nutritional interventions in the short term. Although nutrient supplementation to improve host resistance is well-established, further local studies (particularly in goats and cattle) may be needed to identify best practices for on-farm application and how these can vary between different systems, environments and breeds, including evaluation of protein/energy sources, amount and timing of administration, and their cost-benefit. The immunomodulatory activities of specific dietary compounds during GIN infection remain to be further explored, especially in cattle and goats. The next decades could see leaps in our understanding of diet-microbiota-parasite interactions and an exciting possibility is the identification of gut bacteria naturally synthesising antiparasitic molecules that could be introduced to the GM as live 'anthelmintic factories' [107].

Characterisation of plant resources rich in BC but not yet explored for their anthelmintic potential may reveal novel nutraceuticals of relevance in different settings and production systems. For crops with known bioactivity, a prospect is the selection and breeding of cultivars with high concentrations of BC and enhanced antiparasitic efficacy, which seems realistic based on recent genomic research in e.g. chicory [108]. An alternative could be to isolate natural compounds from plant material for the development of functional feeds with a standardised BC profile, although significant research is needed to characterise the anthelmintic activity and pharmacokinetics of purified plant molecules (individually or in combination) in ruminants *in vivo*. In practice, on-farm implementation of nutraceuticals with proven antiparasitic activities could benefit from modelling studies to determine the timing of targeted feeding, including their influence on parasite establishment, mortality and fecundity following the range of efficacies previously identified in experimental conditions (e.g. [72]).

In the next years, farmers in several countries may have access to commercial *D. flagrans*-based products which will need validation of their cost-effectiveness under different farming settings and climate conditions, while work on developing novel delivery methods of sustained release still is highly warranted. Furthermore, elucidating the ecological interactions between free-living GIN stages and biotic environmental factors can open further options for the biological regulation of parasitic nematodes, and the roles of nematophagous fungi, dung beetles, earthworms and other soil fauna as biocontrol and bioaugmentation in the soil are beginning to be understood [109]. 'Green' or other low-input ruminant farms offer an interesting model for this research due the expected unaltered dung/soil fauna following their lower reliance on veterinary drugs and reduced tillage.

Concluding remarks

Research on a 'green' toolbox for ruminant GIN control can provide relevant non-chemotherapeutic options for integrated parasite management, while advancing our knowledge on fundamental aspects of host–parasite–environment interactions. Notwithstanding, several applied and mechanistic questions warrant further investigation (see Outstanding questions), including the development of innovative research approaches to overcome practical obstacles. For example, determining the optimal efficacy levels of 'green' tools on-farm, including their trade-offs with animal productivity, requires costly, long-term trials that are system/environment-dependent and may only provide locally relevant information, limiting the chances of attracting research funding and that could explain why such studies are scarce. Modelling can help to address this challenge and contribute to the experimental development of non-chemotherapeutic approaches, as well as a support tool to guide the decision on which intervention(s) are the most appropriate to implement locally/seasonally. Pragmatically, integrating elements from the developing 'green' toolbox with targeted, selective anthelmintic treatments can offer a realistic basis to design effective, flexible and customised nematode control strategies for all ruminant farms, aiming to sustain healthy animals and robust farming systems.

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Outstanding questions

What level of 'green' tool efficacy is required to make meaningful impacts on GIN control, ruminant health and productivity in the field?

What are the trade-offs between the individual/combined implementation of 'green' tools for GIN control and animal productivity? Do these trade-offs differ between ruminant species/age groups/production stages? Can we simulate these trade-offs over time?

How can we help farmers to decide which 'green' tools will work best for them locally/seasonally? Can modelling help with this decision? What on-farm data relating to the different 'green' tools needs to be collected to inform mathematical modelling and decision support tools for farmers and advisors?

What is the cost-benefit of applying 'green' tools with limited *in vivo* efficacy versus (not) using anthelmintic treatments with poor efficacy due to drug resistance?

What mechanisms underpin host tolerance to GIN infection? What are the consequences of selectively breeding for host tolerance on animal performance and pasture contamination with GIN over extended periods?

How best to integrate current and future vaccines with other nematode control measures when parasite challenge is high?

How the altered gut microbiota observed in ruminants infected with GIN can impact animal health and productivity? Does the ruminant gut microbiota produce antiparasitic molecules that can be exploited as 'anthelmintic factories' against GIN?

What are the anthelmintic mechanisms of plant BC *in vivo* against GIN? What is the effect of rumen fermentation and compartmentalised gut environments on their bioactivity? Can nutraceutical plants be selected for standardised and higher content of antiparasitic molecules without negatively affecting animal performance and palatability? Can BC from plant material be used to develop functional feeds with antiparasitic activity for ruminants?

What mechanistic/basic data from the different 'green' tools is needed to inform mathematical modelling?

Declaration of interests

The authors declare no competing interests.

Resources

[i]https://ec.europa.eu/eurostat/databrowser/product/page/ORG_LSTSPEC

[ii]https://ec.europa.eu/eurostat/databrowser/product/page/APRO_MT_LSCATL; https://ec.europa.eu/eurostat/databrowser/product/page/APRO_MT_LSSHEEP; https://ec.europa.eu/eurostat/databrowser/product/page/APRO_MT_LSGOAT

[iii]https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:52020DC0380

[iv]https://wormboss.com.au/tests-tools/the-barbervax-vaccine-program/

[v]www.ncbi.nlm.nih.gov/datasets/genome/GCA_964213955.1/

[vi]www.ncbi.nlm.nih.gov/datasets/genome/GCA_963978905.1/

[vii]www.scops.org.uk/forecasts/nematodirus-forecast

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