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Control of helminth ruminant infections by 2030

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Abstract

Helminth infections have large negative impacts on production efficiency in ruminant farming systems worldwide, and their effective management is essential if livestock production is to increase to meet future human needs for dietary protein. The control of helminths relies heavily on routine use of chemotherapeutics, but this approach is unsustainable as resistance to anthelmintic drugs is widespread and increasing. At the same time, infection patterns are being altered by changes in climate, land-use and farming practices. Future farms will need to adopt more efficient, robust and sustainable control methods, integrating ongoing scientific advances. Here, we present a vision of helminth control in farmed ruminants by 2030, bringing to bear progress in: (1) diagnostic tools, (2) innovative control approaches based on vaccines and selective breeding, (3) anthelmintics, by sustainable use of existing products and potentially new compounds, and (4) rational integration of future control practices. In this review, we identify the technical advances that we believe will place new tools in the hands of animal health decision makers in 2030, to enhance their options for control and allow them to achieve a more integrated and sustainable approach to helminth control in support of animal welfare and production.

Introduction

Animal disease control in general, and management of helminth infections in particular, have an important role to play in increasing livestock production to meet future protein needs. This is particularly relevant in the context of a shrinking natural resource base for livestock production and the need to reduce greenhouse gas emissions from the livestock sector to meet internationally agreed emissions targets (Charlier *et al.* 2015; Ozkan *et al.* 2016). Social and economic pressures are likely to demand not just more production, but more efficient and more sustainable production (Thornton, 2010) while also ensuring animal welfare. Given the ubiquitous presence of helminth infections in livestock and their pernicious effects on production efficiency, effective control of these parasites will be key to achieving such aims.

The control of helminth infections in livestock, over the past decades and still today, is primarily based on the preventive or curative use of chemotherapeutics. However, due to their intrinsic genetic diversity, helminth infections have consistently evolved to circumvent existing control measures. As a consequence, we are currently faced with an escalating spread of anthelmintic resistance (AR) and infection patterns that also are altered by changes in climate, land-use and farming practices which can undermine control routines (Skuce *et al.* 2013).

In two recent publications, the key research priorities for helminth control in farmed ruminants and pigs were identified, in order to support the development of roadmaps and strategic research agendas by governments, industry and policy makers (Beesley *et al.* 2017; Charlier *et al.* 2017). These priorities were derived from the DISCONTOOLS gap analysis and follow-up discussions within the recently formed Livestock Helminth Research Alliance (LiHRA, http://www.lihra.eu). We anticipate that, with funded research and stakeholder involvement, these priorities will be addressed in the coming decade and yield new tools for better control. Here, we present a vision of helminth control in farmed ruminants by 2030, bringing to bear the advances in the various fields of control. More specifically, in the following four sections, we will describe challenges and the expected solutions and challenges around: (1) global advances in diagnostic tools, (2) innovative control approaches based on vaccines and exploitation of breeding for resistance and resilience, (3) anthelmintics, with a focus on sustainable use and the likelihood to discover new compounds and (4) the rational integration of future control options.

Diagnostic tools

Global advances in diagnostic tools

Advances in diagnostic tools are a very important issue to better guide the control of helminth infections. A revolution in technology is underway in the diagnostics industry, which will expand in time to veterinary medicine. Macroparasite infections differ from common bacterial and viral infections in that the parasites do not multiply within the host. Thus, the infection intensity (i.e. the number of infectious stages taken up and the size of the established parasite burden) is important, and not simply the state of the animal as infected or uninfected. The most useful diagnostic tools will therefore assess parasite infection intensity levels and also their impacts on key production parameters.

In human health, traditional providers of diagnostic equipment, reagents and services increasingly have to compete with new players that enter the market from a technology background. For instance, through the development of wearable trackers and sensors or devices connected to the internet, it is now possible to continuously collect and analyse data to provide health advice as we aspire to achieve a next level in preventive medicine. A typical example is smart watches that collect information on various parameters such as movement patterns or body temperature and transform these to advise or trigger early warnings to optimize the user's health.

A similar trend is happening in livestock diagnostics, falling within the concept of 'precision livestock farming (PLF)'. PLF is defined as managing individual animals by continuous real-time monitoring of health, welfare, production, reproduction and environmental impact (Berckmans, 2017). Farmers get a warning when something goes wrong in such a way that the PLF system brings them to the animals that need their attention at that moment. The monitoring can be done by camera and real-time image analyses, by microphone and real-time sound analyses, or by sensors around or on the animal (Berckmans, 2017). Well-designed PLF systems enable farmers to manage larger herds in a more time-efficient manner (Rutten *et al.* 2013). In cattle, automated systems already exist to monitor behavioural activities for the detection of lameness and oestrus (Norton and Berckmans, 2017). PLF systems will have to be developed for other important health events, including the management of parasitic disease, and integrated into a single management system for farmers. These systems will make use of advanced technologies like microfluidics, sound analysers, image-detection techniques, sweat and salivary sensing, serodiagnosis, and others (Neethirajan, 2017).

Diagnosis of helminth infections is traditionally based on the detection of worm eggs (or larvae) in feces. However, this is a relatively cumbersome and time-consuming process, since fecal samples have to be shipped to a laboratory where they will be examined by trained personnel. Accordingly, on most farms fecal diagnosis is conducted rarely, if at all. However, recently new technological systems for on-farm sample processing and remote parasite detection have been developed, such as FECPAK^{G2} (Mirams, 2016). In this case, sample processing does not require any specific technical skills, and worm egg identification and quantification are done on-line by trained personnel, who view digitally acquired micrographs. Application of such systems could allow to monitor infection status, reduce treatment costs, and attenuate risks for the development of AR, and thus has great potential to become an important asset in future worm control.

Additionally, several immunological or DNA-based diagnostic techniques have been introduced, increasing sensitivity and cost-effectiveness for detecting the presence and possibly abundance of parasites. These advances have enabled the establishment of semi-automated procedures in diagnostic laboratories, but still require considerable labour input or investment in expensive equipment. The digital revolution must result in a new move towards rapid, cheap and accurate point-of-care diagnostics with correct storage of the data that identify the animals or situations when management intervention is required. Thus, recent years have seen an ever-increasing fidelity of, and decreasing costs for, nucleic acid sequencing methods like second- and third-generation sequencing tools. This progress has already led to new platforms for whole genome and metagenome analyses that are accessible for routine diagnosis in various medical fields, though admittedly not yet for helminth infections in ruminants. Recent developments in nanopore-based third-generation sequencing technology already include portable USB flash drive size sequencing devices like the MinION (Oxford Technologies) sequencer. This, for example, enables on-site mass-sequence data acquisition and has been shown to allow bacterial metagenome analysis (Benítez-Páez and Sanz, 2017). Currently, third-generation sequencing methods still exhibit high error rates, which may be expected to be solved in the future. Furthermore, for merely detecting and differentiating different helminth species, these error rates can be considered to be tolerable. Tools that can accurately quantify levels of parasite infection are perhaps further away, although binary outcomes based on a threshold meaningful to production loss are a possible solution (Mazeri *et al.* 2017). Thus, it can be expected that on-farm multi-pathogen species detection using nucleic acids analysis will become technically and economically feasible even before 2030. Notably, the term 'multi-species' will certainly include bacterial and viral pathogens.

Such on-farm sequencing tools will not only allow the detection and differentiation of the different helminth species infecting individual animals, but also the detection of genetic characteristics associated with AR. However, for most of the presently used drug classes, we still lack genetic markers for resistance (Kotze *et al.* 2014). Thus, our understanding of the basic molecular mechanisms of AR must improve considerably before the information obtained by third-generation sequencing can be assessed meaningfully in the context of AR. This is in particular the case for the mechanism of resistance against the macrocyclic lactones, currently the most frequently used drug class in ruminants. Here, it appears that target site sequence polymorphisms do not play a major role in resistance mechanisms and thus cannot be employed for resistance detection (Kotze *et al.* 2014). The opposite is the case for benzimidazole resistance, for which β-tubulin sequence polymorphisms have been used to develop quantitative resistance assays (Demeler *et al.* 2013). It must be acknowledged, however, that such tests are currently not routinely employed for monitoring ruminant herds; neither is testing for AR using any modality routinely performed. It appears reasonable to predict that this will not change in the future unless testing for AR becomes increasingly cheap and effortless and includes markers for the major resistance mechanisms of all relevant anthelmintic classes.

The proteomic analysis of biological material also has great potential for future diagnostic use in ruminants to detect helminth infections. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling has already been evaluated for the direct or indirect detection of bacterial infections in dairy cattle (Wareth *et al.* 2015; Barreiro *et al.* 2017). Concerning the analysis of parasitic helminths, MALDI-TOF MS has been described for the identification of *Trichinella* spp. (Mayer-Scholl *et al.* 2016) and initial results were also reported for cyathostomins, the most prevalent GI helminths of horses (Bredtmann *et al.* 2017). The advantages of this technology include low costs per sample analysis and reliability. It allows multi-species detection and differentiation by the analysis of a single crude protein extract. However, this technique first needs to be established and validated for the multiple helminth species occurring in ruminants. It seems highly feasible that, similar to the nucleic acid-based tools mentioned above, other important bacterial and viral pathogens will also be detectable in parallel by the analysis of the same sample. However, it is currently unclear if quantitative proteomic analysis of helminth infections on a species level will be possible.

Finally, the detection of biomarkers, such as liver proteins, regulatory hormones or acute phase proteins, has great potential for future health monitoring in ruminants, including in the context of helminth infection (Manimaran *et al.* 2016; Marco-Ramell *et al.* 2016). Advantages of such biomarker detection tools include that non-invasive sample matrices like saliva can be used and that multiple parameters and/or pathogens can be detected in single samples. The latter has, for example, been demonstrated for helminth infections in cattle, for which a magnetic bead-based multiplex assay has been developed for the simultaneous detection of antibodies directed against liver, lung and gastro-intestinal helminths (Karanikola *et al.* 2015). Increased bandwidth of biomarkers in terms of diseases detected is likely to trade off with lower specificity for individual infective organisms, while profiling of multiple disease states might benefit from big data and machine learning approaches. The future may see an extension of such test formats to include relevant other health biomarkers and the use of non-invasive sample material such as milk for routine herd health monitoring.

Translation of new diagnostic technologies to manage parasitic disease on farms

While the above-described laboratory-based tests mostly focus on population-level species diagnosis, there will also be a need for on-farm tests, which can be used as real-time decision tools. In a first step, traditional diagnostic matrices like feces, serum or milk can be combined with novel technologies such as automated image analysis (e.g. using smartphones) and isothermal DNA amplification. This has the advantage of detecting and quantifying helminth eggs or a diagnostic test reaction without the need for expert interpretation of images or results (Slusarewicz *et al.* 2016).

Sensors can also be installed in the environment of the animals to analyse movement, sound or other parameters (Ferrari *et al.* 2010). In this sense, automated weighing scales or assessment of body condition scoring have been evaluated to identify animals requiring treatment for GI nematodes, with varying success (Charlier *et al.* 2014*a*). Potentially, sensors could also be placed in housing or milking facilities to detect pathogens or biomarkers directly in feces or milk (Neethirajan, 2017). For pasture-borne helminth infections, sensor networks could detect weather and environmental conditions on pastures, which combined with predictive transmission models of parasitic disease, can alert producers when pasture infectivity levels exceed certain thresholds (Verschave *et al.* 2016).

Considerable advances are likely to come from sensors and wearable technologies that can be implanted directly in or on the animals, in parallel to the trend discussed above in human health monitoring. Such sensors have already been designed to detect sweat constituents, measure body temperature, observe behaviour and movement, detect stress, analyse sound and detect pH (Neethirajan, 2017). Several concepts that could greatly benefit from these novel technologies have already been explored in the field of helminth control. For instance, GI parasitism is known to alter grazing behaviour and changes in this behaviour, e.g. when detected by location or activity, can be used for diagnostic purposes (Szyszka *et al.* 2013); animal movement or sound analysers could alert for coughing on pasture due to lungworms, or assess which animals have been grazing areas contaminated with liver fluke metacercariae (De Roeck *et al.* 2014), while a wireless pH sensor (e.g. Weinstein *et al.* 2013) in the abomasum could be used to monitor parasitic gastritis and optimize feeding regimens. Diagnostic markers of parasitic infection, currently detectable in milk, saliva or feces (Shaw *et al.* 2013; Charlier *et al.* 2014*b*), will be evaluated in sweat and could reach a next level in ease of use.

To conclude, rapid, cheap and accurate point-of-care diagnostics will enable real-time identification of heavily infected animals, which can then be treated to maintain productivity and/or removed from the breeding population.

Innovative control approaches

Vaccination

Very few helminth vaccines are on the market for livestock. Currently, for nematodes these comprise only the cattle lungworm (*Dictyocaulus viviparus*) vaccine (Bovilis® Huskvac, MSD Animal Health; Jarrett *et al.* 1958) and a vaccine against the barber's pole worm (*Haemonchus contortus)* in sheep (Barbervax®, Wormvax Australia Pty Ltd.), the latter available in Australia and South Africa only (Le Jambre *et al.* 2008). The ongoing development of experimental vaccines against several other helminth species in livestock (e.g. *Teladorsagia circumcincta* in sheep, *Ostertagia ostertagi* and *Cooperia oncophora* in cattle, and the liver fluke, *Fasciola hepatica*, in ruminants) holds promise for a wider range of helminth vaccines in the future (Matthews *et al.* 2016).

Some important technical issues need to be resolved for most of these experimental vaccines before they can be developed into commercial products. Protective native (glyco-)proteins should be 'translated' into protective recombinant or peptide vaccines (Matthews *et al.* 2016). Only two recombinant helminth vaccines, against the cestodes *Echinococcus granulosus* in ruminants (Providean HidatilEG95®, Tecnovax) and *Taenia solium* in pigs (Cysvax®, The Indian Immunologicals Limited) are commercially available. However, based on rapid evolution in proteomics and glycomics technologies, it seems reasonable to expect that additional recombinant or synthetic helminth vaccines will be available by 2030. Similarly, optimization of antigen delivery systems will be driven by increasing knowledge of host–parasite interactions (Matthews *et al.* 2016).

Currently available vaccines against viral and bacterial pathogens are either used in official disease control programmes to contain endemic or epidemic diseases, e.g. bluetongue virus vaccines (Mayo *et al.* 2017), or by individual farmers to prevent disease and associated production losses, e.g. vaccines against neonatal bovine diarrhoea (Meganck *et al.* 2015). It is unlikely that the use of helminth vaccines will be imposed by policy makers, since helminth infections in livestock are considered as 'production diseases', without importance for public health or international trade (Charlier *et al.* 2015). Consequently, the decision to vaccinate will be the farmer's responsibility and among other things will depend on the vaccine's performance and cost-effectiveness in comparison with alternative control measures.

Safety is a major issue for vaccines. The development of a promising human hookworm vaccine based on activation-associated secreted proteins (ASPs) was stopped because of serious allergic reactions in vaccinated pre-exposed individuals (Diemert *et al.* 2012). Several vaccines currently under experimental evaluation for livestock helminths also contain ASPs (*O. ostertagi*, *C. oncophora*, *T. circumcincta)*. No side-effects were observed when calves were vaccinated with a double-domain ASP from *C. oncophora* before turnout on pasture (Vlaminck *et al.* 2015), but it remains to be seen whether or not these vaccines are safe to use in regions with year-long grazing, where animals will likely be infected prior to vaccination. It is encouraging that Nisbet *et al.* (2016) observed no adverse reactions in grazing ewes that were vaccinated with a recombinant *T. circumcincta* vaccine containing ASP.

Vaccine efficacy should be high enough to reduce parasite transmission to a level at which disease and production losses are minimized (Claerebout *et al.* 2003). Since the efficacy of helminth vaccines will probably not reach the same level as current anthelmintics, vaccination may be combined with other parasite control measures, such as grazing management (Matthews *et al.* 2016). The required efficacy and duration of protection will thus depend on the host–parasite system and farm management, both of which are influenced by climate and environment. Short-term protection may suffice for GI nematodes in cattle in regions with a restricted grazing season, such as in Europe (e.g. Vlaminck *et al.* 2015) or when vaccination is combined with grazing management practices that reduce pasture infection levels, such as mowing. A longer vaccine effect would be needed in regions with continuous grazing throughout the year, such as South America and New Zealand (Matthews *et al.* 2016) or where adult animals remain susceptible to (re)infection (e.g. liver fluke). Moreover, immune responses to vaccination may vary greatly between individual animals within a herd or flock (e.g. Nisbet *et al.* 2013; Nisbet *et al.* 2016). In this case, user-friendly diagnostics will be needed to identify animals that respond poorly to vaccination, so they can be treated with anthelmintics or removed from the group.

Future vaccines ideally should protect against multiple helminth species that affect grazing animals with a single product (Matthews *et al.* 2016), either different species of GI nematodes or GI nematodes combined with lungworms and/or liver fluke. However, monovalent vaccines may be useful in situations in which a single parasite species dominates (e.g. *H. contortus* in warmer regions), when other parasites are controlled by alternative measures, or in regions where the risk for other parasites is low (e.g. liver fluke has a heterogeneous spatial distribution). Helminth vaccines could also be combined with vaccines against other pathogens. However, vaccines against multiple pathogens are particularly useful when they tackle a common disease complex, such as neonatal bovine diarrhoea (a combined vaccine against *Escherichia coli*, rotavirus and coronavirus) or bovine respiratory disease (e.g. vaccine against bovine respiratory syncytial virus, parainfluenza virus and *Mannheimia haemolytica*). Parasitic gastroenteritis and husk are well-defined disease entities in grazing ruminants, and therefore combining a GI nematode or lungworm vaccine with bacterial or viral antigens may carry little advantage.

Although ultimately it will be the farmer's decision to vaccinate, the veterinarian remains crucial to implement and improve vaccination strategies (Cresswell *et al.* 2014). Elbers *et al.* (2010) identified economic factors as the main motivators (e.g. increased production) and barriers (e.g. vaccination costs) for farmers when deciding whether to vaccinate their livestock. However, cost was not recognized as a barrier to vaccination by Cresswell *et al.* (2014), and Sok *et al.* (2016) identified farmers' attitudes and social pressure as significant factors driving their intention to vaccinate against bluetongue virus. Identifying drivers of farmers' and veterinarians' decision making will be crucial to optimize uptake of novel parasite control tools. Importantly, drivers affecting the adoption of different worm control measures (e.g. diagnostics, vaccines, targeted selective treatments) may differ between farmer populations (e.g. sheep farmers *vs.* cattle farmers) or countries. For example, the perceived risk for AR had a significant positive effect on UK sheep farmers' adoption of SCOPS guidelines for worm control (Jack *et al.* 2017), while risk perception of AR had no apparent effect on intention to adopt parasite diagnostic methods among Belgian dairy farmers (Vande Velde *et al.* 2015). It must be realized that farmers in resource-poor settings may respond differently to vaccination advice (Heffernan *et al.* 2008), an area which requires more investigation.

By 2030, we expect vaccines against key helminth species in ruminants to be commercially available and to be accepted by veterinarians and farmers as one of the essential tools for sustainable parasite control.

Holistic incorporation of animal resistance and resilience to parasites on farms

For the past four decades, the notion that farmed animals display an inherent, genetically determined, degree of resistance to parasites has attracted substantial research interest (Emery *et al.* 2016). In more intensively farmed systems, exploiting genetic differences at the individual herd/flock population level has been the focus of attention (Woolaston and Baker, 1995). Especially in extensively farmed systems, breed-level inherent resistance and resilience (e.g. the ability of animals to maintain performance in the face of parasitic challenge) to parasites has been studied (Behnke *et al.* 2011). There may well be scope to also exploit breed-associated resilience in intensive systems (Amarante *et al.* 2004). Initially, host selection criteria were based on nematode FECs, shown to have low mean heritability (0·27; Safari *et al.* 2005), but more promising, IgA-based, near-field selection approaches have become available (Shaw *et al.* 2013). Meanwhile, a plethora of molecular markers for host resistance to a variety of GI nematode species has been identified (reviewed by McManus *et al.* 2014) and a framework for the discovery and application of new markers was proposed by Emery *et al.* (2016). Although it is therefore firmly established that resistance to parasites, as well as parasite tolerance, are likely to have an important role to play in future control efforts, bringing established principles to the field remains problematic (McManus *et al.* 2014). As energetic efforts towards mounting strong immune responses trade off with other desirable host traits, notably weight gain and milk production (Walkden-Brown and Eady, 2003), it is not known whether breeding for this trait is always cost-effective. Nonetheless, a recent study in dairy cattle showed negative genetic correlations of milk yield and protein% with endoparasite infection, indicating that genetic progress in both trait categories simultaneously is possible (May *et al.* 2017). On the other hand, breeding for resilience may, through high infection levels at pasture, lead to clinical disease in less resilient hosts within the population (Gibson and Bishop, 2005). For breeding for resilience and/or resistance (RR) to become commonplace on commercial farms, several hurdles must be overcome. An immediate challenge remains quantifying trade-offs between production parameters and immune efforts to facilitate full cost-benefit analyses. A second challenge is balancing RR to macroparasites with immune efforts towards other infectious organisms encountered on intensive farms. The key challenge for 2030 will be embedding RR within a whole-farm approach of optimum energy allocation given their other, farm-specific, energy-demanding 'tasks', animals should be bred to express *optimum* levels of immune efforts (Medley, 2002).

For the farm animals of the future to be able to juggle all energy demands placed upon them, a whole-farm approach needs to be adopted. Energy uptake can be influenced in at least three ways: maximizing uptake capacity, optimizing food quality and maximizing food uptake (opportunities). Energy thus gained will have to service growth (of the immature animal), milk production, reproduction and immune efforts. Traditionally, the effects of one factor on another, for example, lameness and milk production (Green *et al.* 2014), or improved food quality and worm burden (Houdijk *et al.* 2012), have been studied, but a whole-system analysis approach is needed. For example, breeding for resistance may make sense on a farm where consistently high-quality nutrition, i.e. more than covering needs for production, can be supplied but not if lameness cannot be controlled at the same time. To be able to breed the optimum production animal for a particular farm system, a big data approach, analysing a very large number of parameters longitudinally collected from individual animals, is required. This demands a multi-level modelling approach, which is challenging yet achievable utilizing statistical advances and modern computer power.

Anthelmintics

As a consequence of their high efficacy and convenience of use, worm control worldwide has relied heavily on the use of anthelmintics. It may even be stated that since the 1990s anthelmintics have been used more as production tools rather than for diagnosis-guided therapy. Concurrently, increasing wealth in emerging economies has led to increased demand for meat protein in concert with reduced opportunities for traditional non-constrained livestock production due to diminished availability of land for roaming livestock. The convergence of these factors has resulted in the worldwide development and spread of drug-resistant helminth populations that threaten current expectations of positive cost-benefit outcomes for parasite control.

The discovery of anthelmintics in the future

The search for new anthelmintics will continue in the future (Geary *et al.* 2015), expanding to include the use of extracts of medicinal plants. However, recent and probably continuing consolidation of the animal health industry means that fewer and fewer resources are devoted to anthelmintic R&D. New products may be scarcer as a result; however, they likely will be more judiciously deployed.

Empirical research continues to be the foundation of the discovery of anthelmintic agents (Woods *et al.* 2007, 2011; Woods and Knauer, 2010; Geary *et al.* 2015), despite investment in alternative approaches (Geary, 2012). The most successful paradigm in history has been to treat animals infected with parasites with experimental compounds and measure consequent changes in parasite burdens after necropsy. However, screens in infected animals are typically labour-, time- and compound-intensive; consequently, this research strategy has in recent years been almost completely discontinued. In addition, the '3R' principles (Replacement, Reduction and Refinement) are increasingly prioritized as a framework for conducting science in the academic and industrial sectors with higher focus on developing alternative approaches that avoid the use of animals.

Drug discovery in the parasite realm has obviously evolved over the past century. Technological advances resulted in an increased focus on mechanism-based approaches to drug discovery and this is projected to increase as our capabilities advance to improve both the throughput of assays and the quality of data generated (Geary *et al.* 2015). The new wave of screens is based on advances in molecular biology and material handling platforms, and it energized a massive strategic switch in the pharmaceutical industry in the 1990s from phenotypic or whole-organism screens to very high throughput mechanism-based screens, which identify compounds that affect specific protein targets rather than a biological endpoint. These screens have benefited from knowledge discovered about the modes of action of anthelmintics. This knowledge allowed prediction of combinations of drugs that can be used together rationally to increase the spectrum of action and to slow the development of AR (Martin *et al.* 2015). However, the disappointing return on investment in this area has led to renewed focus on high-throughput screening platforms that employ readily available stages of important parasites in phenotypic formats (Geary *et al.* 2015). New developments in phenotypic screening strategies will continue to expand opportunities for anthelmintic discovery. For example, recent experiments have validated the use of *Ancylostoma ceylanicum* L4s and *Ascaris suum* L3s with the microfluidic electropharyngeogram (EPG) platform, providing a new tool for screening anthelmintic candidates or investigating parasitic nematode feeding behaviour. The eight-channel microfluidic EPG chip provided a convenient and powerful new tool for detecting the integrity of electrophysiological signalling in nematodes and its perturbation by applied drugs, compounds or natural products (Weeks *et al.* 2016).

It must be recognized that several new anthelmintic classes have been brought to market for veterinary applications in the fairly recent past, including cyclic depsipeptides (emodepside), amino-acetonitriles (monepantel) and paraherquamides (derquantel), with others presumably in various stages of development (Epe and Kaminsky, 2013). Too few data are available to allow an in-depth analysis of why mechanism-based approaches have not yet succeeded in parasitology (Geary, 2012; Crowther *et al.* 2014). Many challenges limit the ability to conduct mechanism-based screens for antiparasitic drugs, including difficulty in obtaining functional expression of some parasite proteins in assay-friendly formats and extrapolating activity in protein-based assays to whole-organism screens. It remains to be seen if the current dearth of hits derived from these strains reflects poor choice of targets, insufficient investment or a fundamental flaw in the strategy. Learning more about the fundamental biology, biochemistry and physiology of parasites can reasonably be expected to lead to more effective drug discovery efforts. However, the ease of running high-throughput phenotypic screens will continue to foster this unbiased approach into the future, supplemented by 'repurposing' screens in which collections of compounds validated for activity against known molecular targets in other organisms (insects or mammals) are screened against parasitic helminths, potentially facilitating the discovery of new useful compounds for this indication.

Future of natural products working against helminths

Direct anthelmintic properties of bioactive forages, e.g. plant cysteine proteinases, flavonoids and condensed tannins, have been confirmed *in vitro* and *in vivo* in small ruminants and cattle and more than 850 papers were published on the use of natural compounds the last 20 years. Klongsiriwet *et al.* (2015) showed the first evidence of synergistic effects between condensed tannins and two common flavonoids, quercetin and luteolin, in terms of inhibiting the *in vitro* ex-sheathment of *H. contortus* L3s and recently Doligalskaa *et al.* (2017) indicated that *Avena sative*-originated saponins may represent an inexpensive source of anti-parasite natural products. These findings suggest that opportunities should be investigated for increasing anthelmintic activity by mixing plant materials that contain condensed tannins and quercetin or luteolin flavonoids, or to select plants with enhanced tannin and quercetin or luteolin contents. It is likely that many natural products, even in crude mixtures, act on pathways in worms that differ from targets of currently used anthelmintics and therefore might kill nematodes that are resistant to one or more existing anthelmintics.

Bioactive forages can also be used as part of the diet that deliver both anthelmintic and nutritional benefits due to the presence of plant secondary metabolites with anthelmintic activity. As such, they fall under the concept of nutraceuticals (Hoste *et al.* 2015) – defined as 'any substance that may be considered a food or part of a food which provides health benefits, including the prevention and treatment of disease' – although some may eventually be developed as stand-alone drugs. The development of nutraceutical products with real potential for the control of helminths in ruminants is a possibility that is well on the way to becoming reality in different parts of the world in various livestock breeding systems.

However, for the vast majority of such natural compounds, there have been limited systematic, scientific evaluations of efficacy, mode of action and identity of their active component and no plant-based anthelmintic is yet commercially available. Bottlenecks for a more widespread use include difficulties in registration, unknown mode of action, possible presence of several important secondary bio-actives as interacting metabolites, toxicity due to the presence of other uncharacterized secondary metabolites, residues, difficulties in quality assurance, and challenges in manufacturing and distribution.

The use of anthelmintics by 2030

We will still depend on anthelmintics in 2030; however, we anticipate that anthelmintics will be used far more selectively. Innovative control approaches and better diagnostics will have become available that allow treatment to be targeted only to those animals that need it to support optimal health and production, and at the right time. We will thus be entering a new anthelmintic era.

It must be recognized that, if anthelmintics are used more selectively and fewer doses are sold, this could reduce the incentive for investment in discovery. On the other hand, targeted therapy may allow the introduction of more costly products, because the expense of treating selected animals will be no more than that of current number of 'wasted' doses (treatments given to animals that do not benefit from them). An anticipated benefit of a change from preventive to therapeutic or highly targeted treatments is that AR will develop less quickly against new anthelmintics, thus preserving a longer period of efficacy. Environmental impacts of treatment are also likely to reduce (Cooke *et al.* 2017).

It is also important, from an industry perspective, that future anthelmintic products (mono-active or multiple active) will be framed within a management plan. It is likely that regulators will impose constraints on the use of anthelmintics in the future to limit environmental and food residues, restricting the use of anthelmintics only after diagnosis has shown the presence and/or importance of helminth infections (Charlier *et al.* 2017). A regulatory environment that promotes best-practice parasite management recommendations and prohibits the use of anthelmintics with low efficacy may stimulate future innovation in the field of therapeutics.

To conclude, by 2030 we can expect to have new anthelmintics and/or anthelmintic combination products and we will see a major change in the way worm control is approached in ruminants. The availability of new diagnostic tools will result in more targeted treatments, while vaccines will become available and highly heritable immune biomarkers will be discovered and used in future selection programmes. Reliance on whole-herd routine anthelmintic use will therefore be reduced. Of course, new tools will only truly find their way to farms if they are communicated to farmers and vets in the appropriate manner, and make economic sense. Improved involvement of these stakeholders will therefore have to underpin all future control efforts.

Rational integration for future control practices

Future considerations for integrated decision-making

In the past, decision-making on helminth control has focussed on reducing parasite burdens and improving productivity. The fact that negative production impacts of parasitism should be reduced was taken for granted, and the main questions addressed were thus how novel control approaches could be developed with a higher or more persistent efficacy or ease of use. Currently, increasing importance is given to the impact of livestock production on the environment and animal welfare (Godfray *et al.* 2010; Niamir-Fuller, 2016). Livestock production activities affect the environment through the use of natural resources as an input, while the by-products of livestock production may cause pollution and other negative impacts on natural ecosystems. The cost of such side-effects of the economic activity of farming is not fully factored into the prices paid by producers or consumers. These side-effects affect the welfare of, or the opportunities available to, individuals or groups without direct payment or compensation (Rushton and Bruce, 2017).

Helminth control can make a positive contribution to these challenges. Liver fluke in cattle, for instance, was estimated to increase greenhouse gas emissions per affected cow by 10% (Williams *et al.* 2015). Anthelmintic control strategies have been shown to reduce farm-level greenhouse gas emissions (Kenyon *et al.* 2013). The effect of helminth control on water use remains to be assessed but is likely to be beneficial, given the vast water requirements for maintenance and production (Ridoutt and Hodges, 2017) and the negative impact of helminths on input–output conversion (van der Voort *et al.* 2014). On the other hand, there are undesired side-effects of helminth control such as labour input, AR development and leakage of anthelmintic residues in the environment (Cooke *et al.* 2017) or food products (Kang *et al.* 2017).

Future decision making on helminth control will increasingly take into account all elements involved. Whereas current economic evaluations provide cost assessments of helminth disease (Fanke *et al.* 2017) or cost-benefit analyses of specific helminth control interventions at farm level (Charlier *et al.* 2012), methodologies are under way to factor in indirect effects in deciding if and how to intervene against endemic helminth infections.

Van der Voort *et al.* (2013, 2017) developed a concept to place GI nematode infection and its potential control measures in the whole-farm economic context. This concept is based on the production function framework (Coelli *et al.* 2005) and allows to link diseases and mitigation strategies to input and output uses of a production system, and to benchmark the performance levels of a farm against its peers. These methods will increasingly support helminth decision-making at the individual farm level. Governments or regulators can also use these methods because they allow calculation of impacts without a known market value, provided that a standardized measure of the variable is available (Rushton and Bruce, 2017). Scores to evaluate environmental performance or animal welfare are increasingly used and accepted. Currently, regulators assess market authorization of a product mainly based on criteria evaluating safety, quality and efficacy. The new methods will allow inclusion of a wider set of criteria that consider the positive or negative impacts of new anthelmintic products on ecological footprint or animal welfare.

The use of these methods greatly depends on scientific data, which are still lacking, or need confirmation in different areas or production settings (Charlier *et al.* 2017). It is only by incrementally growing our understanding of the economic, social and environmental impacts through experiments and surveys and integrating these in whole-system approaches that intervention strategies can be optimized in a holistic manner.

Modelling

Because of the huge variability between geographical and socio-economic farming environments and between the epidemiology of different parasites, and given the availability of multiple control measures, modelling will become indispensable to predict and communicate the outcome of different treatment options. The use of socio-psychological models to understand farmer behaviour with respect to parasite control decisions (e.g. Vande Velde *et al.* 2015; Jack *et al.* 2017) and to design effective communication strategies will support different stakeholders in embracing the new technologies and choices to be made. Modelling parasite transmission patterns (e.g. Rose *et al.* 2015; Verschave *et al.* 2016) and the effects of intervention strategies on parasite epidemiology and production measures such as calf growth (e.g. Berk *et al.* 2016*a*) and on farm economy (van der Voort *et al.* 2017) will help end-users to integrate parasite control into whole farm management.

Predictive models can be used in three ways: (i) understanding fundamental processes that pertain to a large number of systems in a general and transferable way; (ii) determining how interactions in a particular system give rise to disease patterns, and how they might be manipulated by targeted interventions; and (iii) taking inputs from a specific farm to generate specific recommended actions for parasite control. Progress has been made in each field and is likely to accelerate with increases in computer power, establishment of PLF, and flows of data from monitoring of livestock and their health. Computer models are therefore likely to be more prominent than in the past as guides to sustainable parasite control on farms.

At the fundamental level, mathematical models have led to significant advances in our understanding of areas such as parasite population dynamics (e.g. Anderson and May, 1978), and will no doubt lead to further insights in problematic areas. For example, Medley (2002) generated a theoretical framework to explain how allocation of nutritional resources to competing functions of growth, reproduction and immunity means that tolerance of some parasite burden is optimal. This provides a general framework to better understand nutrition–parasite relationships, for example, the periparturient rise in fecal nematode egg counts in sheep (Kidane *et al.* 2009). The general finding that immunity to parasites is not necessarily maximized to achieve optimal productivity has entered system-specific models in livestock (Berk *et al.* 2016*a*) and been applied to interventions such as breed selection (Kidane *et al.* 2010).

When general models are extended to make them more realistic in farm settings, invariably the increased complexity is a barrier to identifying analytic solution (Verschave *et al.* 2016). Results are then often generated by computer simulation, and the consequences of different scenarios and assumptions on outcomes of interest compared. Because helminth transmission is inherently spatial, with movement of groups of livestock between pastures (and between pastures and housing) driving infection in combination with actions of climate on the free-living stages, such simulations must make assumptions on 'typical' farm management. For example, a simulation model of nematode populations on UK farms (Learmount *et al.* 2006) was adapted to Canadian farms (Guthrie *et al.* 2010), taking into account differences in climate and management. Further adaptation would be needed to evaluate future farm conditions, though it would be harder to justify the complex assumptions necessary for this (Kipling *et al.* 2016). Similarly, extension of simulation models to consider specific problems such as AR (e.g. Dobson *et al.* 2011; Learmount *et al.* 2012; Cornelius *et al.* 2016; Berk *et al.* 2016*b*) must be couched in assumptions around farm practice, which can be restrictive, as well as the genetics of AR. Nevertheless, models with this level of detail are needed to make sense of how new tools for control might be best applied and to what realistic ends. This can save a great deal of practical work and expense, for example, by setting desired targets for FEC reduction from vaccines and bioactive forages, or by helping to select and design the most efficient experiments to provide empirical proof of key model outcomes – which will always be needed to convince end-users that model outputs are more than illusions.

While typical farm management patterns can be characterized for a particular system and region, variation can be large between farms and between years, and responsive to factors that are incompletely characterized or understood. Outputs of general models might therefore guide strategies, but be insufficiently attuned to the situation of many farms to be useful for direct decision support at farm level. The key adaptation enabling the application of models to on-farm parasite control by 2030 will be the combination of computer power with new data streams from high-throughput diagnostic tests and sensors. In many cases, automation will be necessary: even manual harvesting and input of basic information on animal movements between pastures is too onerous and unreliable on most farms. Automated collection and streaming of data into parasite models will be crucial to their performance. The presentation of outputs in a meaningful way alongside other drivers of relevant management should also receive more attention. For example, decision support systems for optimal grass utilization in paddock grazing systems might lend themselves to an added parasite management component.

A vision for 2030 is full integration of parasite population models with production and management, calibrated by real-time on-farm automated data collection. Outputs will be generated with minimal additional effort and delivered to farmers and advisors to optimize control practices for production and sustainability.

Implementing decision tools

Implementing many of the advances discussed above will require integration of new tools within changing internal, i.e. farm management, and external (e.g. climatic) contexts. To do this successfully will require unprecedented consideration of host–parasite interactions beyond established experience, and it is unlikely that such understanding can be derived entirely through empirical studies. Thus, for example, the impact of a new candidate vaccine on parasite transmission is likely to differ according to efficacy, stocking rate, inter-current administration of anthelmintic drugs, production systems, and climatic variation within and between years and geographically. It is hard to conceive of a practical trial that could even begin to document how consequences of vaccination play out across this wide and multidimensional parameter space, much less optimize it. Therefore, *in silico* approaches are essential to explore interactions between elements of farm–host–parasite systems, and the consequences of change.

Integration of parasite control into whole farm management will be challenging and should take into account novel technologies, the socio-economic context at farm and societal levels, the environment and animal welfare. These factors will influence, and be influenced by, the management decisions of the farmer. Farmers' expectations of new tools, which involve investment in time, costs or training, must be realistic. At the same time, researchers must consider how the 'entry level' of new technology might be lowered to improve accessibility, either by more research on the tools themselves, or on farmer adoption. To have global impact, this field must expand to embrace the diverse contexts of livestock farming, including in poorer countries and sectors.

Conclusions

Undoubtedly, the control of helminth infections in grazing ruminant animals by 2030 will be different compared with now: we will potentially rely less on anthelmintics because of the availability of vaccines and other control tools such as bioactive forages. As breeding programmes make ground, helped by new markers for optimal phenotypes, ruminants will also become more resilient and resistant to helminth infections. A more rational integration of control practices will be better supported by new diagnostic technologies and interpretation of live information streams, and hence more widely adopted, such that anthelmintic treatment becomes a rescue for underperforming animals rather than a 'blind' whole-group management routine. Removal of 'rescued' animals from the breeding pool will further strengthen resilience. Reduced selection pressure for AR might help reverse the current trend of diminishing efficacy horizons of new products. Nevertheless, new control approaches are unlikely to make anthelmintics obsolete, and the continued discovery of new drugs in more difficult economic circumstances will require a reframing of investment decisions.

A contrary scenario sees production efficiency as king, given increased demand for animal-derived products, a drive for greenhouse gas reduction, and increased competition for land. In that case, regular whole-group anthelmintic treatment of grazing animals could remain economically viable and a staple routine, but will inevitably be undone by accelerating development of AR. Emphasis on production efficiency could increase housing of ruminants and zero-grazing systems; however, grazing will remain important within the production cycle as a key feed resource, and parasite control will therefore remain vital. It is impossible to predict the future of helminth control without considering the likely economic and management context of future ruminant production systems (Kipling *et al.* 2016). Moreover, the drivers of system change are likely to vary widely across the world (Thornton, 2010), affecting the need for and uptake of new technology. Therefore, in this critical review, we have identified the technical advances that we believe will place new tools in the hands of animal health decision makers in 2030, to enhance their options for control and achieve a more integrated, balanced and sustainable approach to helminth control in support of production. Providing high-quality diets to a growing global population without environmental destruction depends on their success.

There are still many challenges ahead but considering (1) the important role of increasing livestock production to meet future needs of high-protein foods from a shrinking natural resource base, (2) the escalating spread of AR and (3) infection patterns that are altered by a changing climate and associated land use and farm husbandry changes, the successful implementation of these innovations in worm control is needed. While scientific progress will yield many future options for helminth control, their integration and adoption should feature centrally in research programmes if benefits are to be fully realized.

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