CHAPTER THREE

# Anthelmintic resistance in ruminants: challenges and solutions

**J. Charlier[a], D.J. Bartley[b], S. Sotiraki[c], M. Martinez-Valladares[d], E. Claerebout[e], G. von Samson-Himmelstjerna[f], S.M. Thamsborg[g], H. Hoste[h], E.R. Morgan[i], and L. Rinaldi[j,*]**

[a]Kreavet, Kruibeke, Belgium
[b]Disease Control, Moredun Research Institute, Penicuik, United Kingdom
[c]Veterinary Research Institute, Hellenic Agricultural Organisation ELGO-DIMITRA, Thessaloniki, Greece
[d]Instituto de Ganadería de Montaña (CSIC-Universidad de León), Departamento de Sanidad Animal, León, Spain
[e]Ghent University, Faculty of Veterinary Medicine, Laboratory of Parasitology, Merelbeke, Belgium
[f]Institute for Parasitology and Tropical Veterinary Medicine, Veterinary Centre for Resistance Research, Freie Universität Berlin, Berlin, Germany
[g]Veterinary Parasitology, University of Copenhagen, Frederiksberg C, Denmark
[h]INRAE, UMR 1225 IHAP INRAE/ENVT, Toulouse University, Toulouse, France
[i]Institute for Global Food Security, Queen's University Belfast, Belfast, United Kingdom
[j]University of Naples Federico II, Unit of Parasitology and Parasitic Diseases, Department of Veterinary Medicine and Animal Production, CREMOPAR, Napoli, Italy
*Corresponding author: e-mail address: lrinaldi@unina.it

## Contents



*Advances in Parasitology*, Volume 115
ISSN 0065-308X
https://doi.org/10.1016/bs.apar.2021.12.002

## Abstract

Anthelmintic resistance (AR) is a growing concern for effective parasite control in farmed ruminants globally. Combatting AR will require intensified and integrated research efforts in the development of innovative diagnostic tests to detect helminth infections and AR, sustainable anthelmintic treatment strategies and the development of complementary control approaches such as vaccination and plant-based control. It will also require a better understanding of socio-economic drivers of anthelmintic treatment decisions, in order to support a behavioural shift and develop targeted communication strategies that promote the uptake of evidence-based sustainable solutions. Here, we review the state-of-the-art in these different fields of research activity related to AR in helminths of livestock ruminants in Europe and beyond. We conclude that in the advent of new challenges and solutions emerging from continuing spread of AR and intensified research efforts, respectively, there is a strong need for transnational multi-actor initiatives. These should involve all key stakeholders to develop indicators of infection and sustainable control, set targets and promote good practices to achieve them.

## Abbreviations

**AH** anthelmintics
**AR** anthelmintic resistance
**GIN** gastrointestinal nematodes
**RUM** ruminants

## 1. Concerted action for combatting anthelmintic resistance in ruminants

Cattle, sheep and goats are parasitized by various helminth species, including gastrointestinal nematodes (GINs), lungworms and liver flukes. These pathogens can cause severe disease, affect productivity in all classes of stock, and are among the most important production-limiting diseases of grazing ruminants globally (O' Brien et al., 2017). Essentially, all herds/flocks in a grass-based production system are affected, and the major economic impact is due to sub-clinical infections causing reduced growth and meat/milk/fibre production. A recent comprehensive study investigated the economic burden of helminth parasitism on the ruminant livestock industry in Europe and found a staggering annual loss of € 1.9 billion, of which the largest share (38%) was caused by GIN infections (Charlier et al., 2020).

A major constraint on the control of helminth infections in livestock is treatment failure due to anthelmintic resistance (AR). Frequent, indiscriminate and/or inappropriate use of anthelmintic drugs to control these parasites has resulted in the selection of drug-resistant helminth populations. AR is now widespread in all the major GIN of sheep, goats, and cattle and is also an emerging problem in liver fluke (Fairweather et al., 2020; Rose Vineer et al., 2020). Anthelmintics make a significant contribution to the maintenance of animal health, welfare and productivity in pasture-based livestock industries, and the scarce development of anthelmintics with a new mode of action in the foreseeable future urges for action to make the use of currently available control options more sustainable (Vercruysse et al., 2018).

In an effort to combat anthelmintic resistance in ruminants, a COST (European Cooperation in Science and Technology) Action named "CA 16230—COMBAR—COMBatting Anthelmintic Resistance in Ruminants" was launched (Box 1). It aims to strengthen collaboration in the development of innovative diagnostic tests to detect helminth infections and AR, sustainable anthelmintic treatment approaches and the development of complementary control approaches such as plant-based control to reduce the need to use anthelmintics. COMBAR also aims to further the understanding of socio-economic drivers of anthelmintic treatment decisions to support a broad behavioural shift by the ruminant health community and its stakeholders and promote the uptake of novel,

### BOX 1 COST Action COMBAR: coordinating research to combat anthelmintic resistance in ruminants.

COMBAR (CA16230) (www.combar-ca.eu) is a running COST Action (23 June 2017–18 March 2022) that emerged from activities of the Livestock Helminth Research Alliance (LiHRA) and aims to advance research on the prevention of anthelmintic resistance (AR) in helminth parasites of ruminants in Europe and disseminate current knowledge among all relevant stakeholders. Whereas previous research has arguably developed disparate approaches to tackle AR, COMBAR aims to promote more holistic approaches considering current developments in the fields of diagnostics, targeted (selective) treatment (TT/TST) approaches, vaccines, anti-parasitic forages and decision support as well as economic and sociologic methodologies to understand economic aspects and barriers to uptake by end-users. Over 200 participants from 31 COST countries, and 3 COST Near Neighbouring Countries are contributing to the Action.

A COST Action is based on a joint work programme lasting four years and implemented through a range of networking tools, such as meetings, conferences, workshops, short-term scientific missions (STSMs), training schools, publications, and dissemination activities. Emphasis is placed on activities involving early career investigators, and researchers from less-research-intensive countries (the so-called Inclusiveness Target Countries; ITCs) with a view to increase their participation and research activities. COST Actions promote the circulation of researchers within Europe, enhance their career development opportunities and limit brain drain from peripheral regions to more research intense regions in Europe (www.cost.eu).

So far, COMBAR organised three conferences in Warsaw (2017), León (2018) and Ghent (2020), respectively and a fourth online (December 2020) due to the COVID pandemic. Similarly, specific workshops, dedicated to one or more working groups (WGs) were organised, two of them physically (Scotland and Northern Ireland) and one online for the same reasons.

Nineteen STSMs have been funded so far, most were focused on learning or getting access to different diagnostic techniques (e.g. FLOTAC system, digital droplet PCR, *in vitro* screening for AR), and on innovative, sustainable control methods (e.g. implementation of TST approaches and modelling interactions between different control approaches). There were no STSMs in the field of socio-economic research activities and this indicates that this research field needs further promotion to get traction in the field of veterinary parasitology.

Promotion of the socio-economic research activities was undertaken via a training school on research methods and practices in this discipline, organised in Bruges (Belgium). A second training school (Naples, Italy) introduced young researchers to FEC methods, FECRTs, *in vitro* tests as well as DNA-based methods

**BOX 1 COST Action COMBAR: coordinating research to combat anthelmintic resistance in ruminants.—cont'd**

for the diagnosis of helminth infections and AR. This TS also contributed to the harmonisation of diagnostic methods and the use of composite samples across laboratories and the development of the COMBAR protocols to conduct FECRTs for research purposes in sheep, goats (Annex 1 in the online version at https://doi.org/10.1016/bs.apar.2021.12.002) and cattle (Annex 2 in the online version at https://doi.org/10.1016/bs.apar.2021.12.002). The third training school was organised online and introduced the participants in the use of computer models to explore the epidemiological effects of TST and complementary control approaches across different farming systems and climates.

evidence-based solutions. The aim of this review is to assess the state-of-the-art in (i) the diagnosis of helminth infections and AR, (ii) sustainable anthelmintic use, (iii) complementary control approaches and (iv) human socio-psychological factors that drive anthelmintic use and the application of new approaches to helminth management to identify sustainable pathways to address the current AR problem. We focus on AR in GIN, where most knowledge is available and draw attention to the areas where COMBAR is most actively coordinating research efforts.

## 2. Prevalence and impact of anthelmintic resistance

### 2.1 Prevalence

Understanding the true occurrence of AR is difficult, with initial reports of resistance generally only being made when clinical signs of parasitic gastro-enteritis have been observed by farmers and investigated (Bartley et al., 2021; Sargison et al., 2001). Initial reports of resistance are generally restricted to a single anthelmintic class and a single parasite species. However, multispecies resistance (i.e. involving more than one parasite species) is increasingly reported and multi-drug resistance (MDR) (i.e. resistance to multiple classes of compounds) has been identified in various studies in all of the economically important GINs of livestock ruminants, in particular *Haemonchus contortus, Teladorsagia circumcincta, Cooperia* species, *Ostertagia ostertagi, Nematodirus battus* and *Trichostrongylus colubriformis*

(Conder and Campbell, 1995; Jackson and Coop, 2000; Jabbar et al., 2006). Systematic reviews and meta-analyses of available prevalence studies were first undertaken in 2015 and updated in 2020 (Rose et al., 2015, Rose Vineer et al., 2020) to record the distribution of AR in the major GINs affecting sheep, goats and cattle across Europe. The latter study revealed that AR in GIN was widespread in Europe, being reported in five economically important GIN genera and across 16 European countries. Aggregated results revealed an average farm level prevalence of AR to benzimidazoles (BZ) of 48 and 51%, to macrocyclic lactones except moxidectin (MLs) of 29 and 44%, to levamisole (LEV) of 32 and 20%, in sheep and goats, respectively and to moxidectin (MOX) in sheep of 17% (Rose Vineer et al., 2020). In cattle, aggregated farm level prevalences of AR across the European continent were 8% for BZ, 32% for ML, 12% for LEV and 27% for MOX. In cattle, both *Cooperia* spp. and *O. ostertagi* were implicated. Furthermore, MDR was confirmed in sheep and goats in 10 European countries, but is likely to be more widespread than reported (Rose et al., 2015).

To get an un-biased impression of the actual state of play it is necessary to conduct a correctly powered study with randomly selected, clearly defined farms from across the geographical area of interest. Such surveys are rare to be undertaken due to the cost, time, resources, and the need for farmer consent and participation. As such, non-random convenience sampling is commonly done, which provides an idea of the situation, but results need to be viewed with caution, particularly in the early stages of resistance development (Sangster, 1999).

Furthermore, reports and prevalence studies are only one part of the story, and can overlook the fact that an efficacy of less than 90%–95% (WAAVP guidelines definition of resistance; (Coles et al., 1992)) does not necessarily mean that a product is ineffective on a farm. Resistance identification at one time of year, in one species, does not preclude the fact that a compound may have utility at another time of the year against a different parasite species. However, it is considered an early sign that the AR situation may worsen if no action is undertaken to slow down its development.

## 2.2 Impact

AR is multi-faceted in its impact, having an influence on the livestock and their keepers, as well as the financial viability and sustainability of enterprises and the environment. Some of the impacts are highlighted in Table 1. Production losses directly attributed to AR are difficult to accurately assess

**Table 1** Potential impacts associated with anthelmintic resistance in livestock ruminants

| Livestock | Profitability | People | Environment |
|---|---|---|---|
| • Impacts on health<br>• Impacts on welfare | • Decreased productivity<br>• Reduction in long-term sustainability of food production<br>• Increased resource requirements / inputs | • Negative impact on health and wellbeing of producers?<br>• Financial pressures<br>• Consumer concerns about chemical usage in agriculture | • Increased greenhouse gas contributions from poor producing livestock<br>• Increase in chemical administrations when initial treatments fail with potential impacts on dung and aquatic fauna |

but studies in adult and young sheep (Macchi et al., 2001; Miller et al., 2012; Sutherland et al., 2010; West et al., 2009) and cattle (Borges et al., 2013; Candy et al., 2018; Fazzio et al., 2014; Sargison et al., 2010) have reported losses in productivity, e.g., ill thrift, slower weight gain, decreased carcass quality/value and reduced milk yields.

Again, additional costs which are often overlooked may include veterinary intervention, the purchase and provision of additional feed stuff to ensure fattening of animals at allotted times, reduced market prices and potentially additional time spent dagging affected animals to prevent fly strike (Sutherland et al., 2010). There are many ways of assessing the financial implications of AR, firstly there are the purely practical losses incurred with the increased costs of buying and administering treatments, of the € 1.8 billion lost to GIN infections around 19% was attributed to treatment costs (Charlier et al., 2020). If combination treatments are required, the costs increase accordingly. Additionally, less easily defined costs include time spent planning alternative strategies, investigating and sourcing alternative compounds, purchasing anthelmintics, gathering and returning stock, constructing and maintaining handling facilities and treating animals. Finally, there is the ultimate cost, total failure, or severely reduced efficacy of all available treatments: fortunately these cases are rare but have been reported in some sheep producing areas in South Africa (Van Wyk et al., 1997), northern and southern America (Terrill et al., 2001; Waller et al., 1996) and the UK (Blake and Coles, 2007; Sargison et al., 2005), leading to some producers finding it economically unviable to continue farming. Moreover, once AR is present on a farm it can develop very quickly, such as shown by El-Abdellati et al. (2010) on a Belgian cattle farm, where faecal egg count

(FEC) reductions following treatment evolved from 73% to 0% over a 3 year period. Model outputs suggest that ML-resistant parasitic worms alone cost the European livestock industry more than €38 million, in production losses and treatment costs annually (Charlier et al., 2020). These total costs estimated across 18 countries were largest in cattle (€ 26.5 million; milk and beef) followed by sheep (€ 10.5 million; milk and meat) and dairy goats (€ 1.1 million). Losses were greatest in the dairy cattle and meat sheep sectors. If the estimated costs associated with ML resistance in ruminant livestock sectors in different climatic zones (based on aggregated Köppen-Geiger categories) in Europe and near neighbour countries are calculated it is possible to see that the impact in Atlantic zones (Belgium, France, the Netherlands, Republic of Ireland and UK) were significantly greater than in Mediterranean zones (Israel, Italy, Portugal, Spain, Tunisia); € 18.0 million and € 7.8 million for cattle and small ruminants compared to € 4.1 million and € 1.1 million respectively, highlighting the differences in scales of losses across Europe and near neighbour countries.

The social and psychological impact of exotic/epidemic and endemic disease incursions on farmers' and veterinarians' well-being is an area of increasing concern. Whilst there appears to be strong evidence that exotic incursions (Boyce et al., 1984, 2021; Phythian and Glover, 2019), and how subsequent control strategies are portrayed and executed, impact on farmer's health and well-being (Boyce et al., 2021), there is little empirical evidence with respect to endemic diseases such as GIN infections. A study in dairy farmers with or without bovine tuberculosis outbreaks in their herds suggested that there was mixed evidence that animal disease impacted on farmer well-being and that stressors are complex and multifactorial (Crimes and Enticott, 2019). The work also suggested that with chronic endemic diseases in their herds/flocks, farmers may become fatalistic and learn to live with the disease, bearing ongoing losses; this outcome may be the case with GIN infection and AR.

Anthelmintic resistance has the capability to impact on the environment in a number of ways such as poor animal performance (live weight gain), as a result of poor parasite control, meaning longer finishing times, increased feed requirements (either concentrates or time on pasture) to fatten up and/or more chemical interventions. Poor productivity and/or longer finishing times, associated with livestock diseases, have been correlated with increased green-house gas emissions intensities (Bartley et al., 2016; Ezenwa et al., 2020; Fox et al., 2018; Kenyon et al., 2013; Kipling et al., 2021). Indiscriminate use of anthelmintic products may influence the biodiversity of invertebrates on pasture and/or in water courses (Forbes, 2021). Environmental impacts associated with anthelmintic usage can be mitigated through the optimisation of anthelmintic administrations in conjunction with the adoption of sustainable/*refugia* based control strategies (Cooke et al., 2017).

## 3. Gastrointestinal nematodes: current and future diagnosis

Controlling nematodes efficiently and sustainably requires insights in the scale of the issue at various levels: regionally, at farm, individual fields or paddocks, and at herd and individual animal levels. There are numerous benefits of diagnosing and monitoring GIN infections and anthelmintic efficacy, including a) identifying the potential causative agent of a health issue; b) building a picture of the parasitological scenario on farm; c) improving livestock production; d) saving money on wasted or ineffective treatments; e) detecting anthelmintic inefficacy or resistance issues to take timely action; f) optimising treatment timing and informing product choice. Here, we outline available diagnostic methods and markers for the detection of GINs in ruminant livestock, while current methods for the diagnosis of AR diagnosis are covered in the next section.

### 3.1 Diagnostic markers of GIN infections

Current diagnostic markers of GIN infections and parasitic gastro-enteritis are broadly based on parasitological (e.g. FEC, peanut agglutinin egg staining, coproculture, Baermannisation), immunological (e.g. parasite specific antibodies in serum or milk), pathophysiological (e.g. ocular mucous membrane colour measured by FAMACHA, packed cell volume, plasma/serum pepsinogen, diarrhoea/dag score) and performance based (e.g. live weight gain, body condition score) indicators (Fig. 1; reviewed more fully by Kenyon and Jackson, 2012).

The usefulness of parasitological diagnostic tests as markers of parasite burden varies due to the influences of factors such as prior exposure, fecundity of parasite species and variation in egg output associated with individual host animals (Gasbarre et al., 1996). The correlation between FEC and worm burden is generally considered poor, particularly in older cattle, but parasitological indicators can be useful to confirm the presence/absence of parasitic nematodes, provide information on parasite type present (nematodes, trematodes and cestodes), to assist in targeting anthelmintic

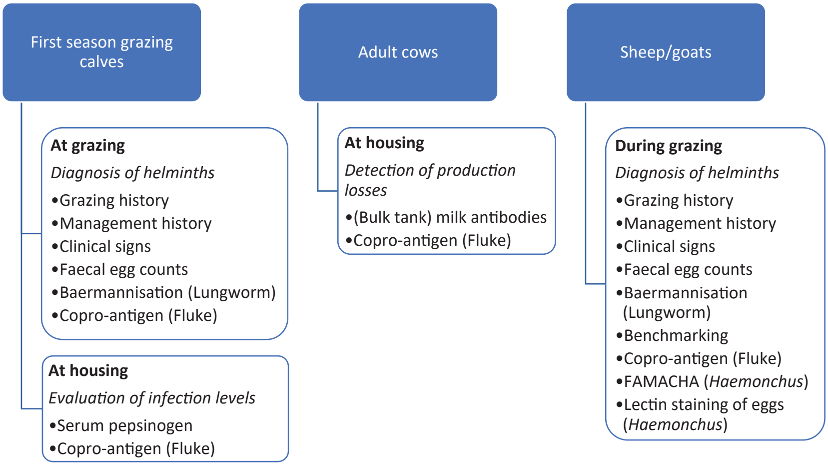


**Fig. 1** Diagnostic markers and their use to assess helminth infection status and associated impacts in ruminants.

administrations or to test the efficacy of products (for which, see Section "Diagnosis of AR" below). Carbohydrate specific binding of the lectin peanut agglutinin is used commercially for the detection and identification of *H. contortus* eggs. Unfortunately, examination of a wide range of other lectins has failed to identify any other with the required specificity to characterise additional nematode species (Umair et al., 2016).

Parasite-specific antibody detection can identify and categorise (sub-) clinical cases of nematode infection but measures mainly previous exposure rather than current infection. Moreover, test results can be influenced by non-parasite related factors such as the age of the animal, milk yield or mastitis. The existing markers can also lack specificity (Charlier et al., 2014).

Pepsinogen assays show good correlation with *O. ostertagi* burden in first season grazing cattle but the correlation wanes in older grazing animals or post housing (Charlier et al., 2014). Reproducibility and harmonisation between labs are a further factor that hampers the pepsinogen assay's reliability and routine uptake (Charlier et al., 2011). More recently leptin and ghrelin have been examined (Forbes et al., 2009), but the profile of leptin concentration in infected calves requires further validation before being considered as a potential pathophysiological marker of parasite induced inappetence. The FAMACHA chart, which uses peri-ocular mucous membrane colour to indicate low packed red blood cell volume, i.e., anaemia, has been used globally to great success in the detection of *Haemonchus* infections in sheep and goats (Van Wyk and Bath, 2002) and more recently also for assessing *Fasciola hepatica* infections (Olah et al., 2015), while results in cattle are more equivocal (Dorny et al., 2011; Grace et al., 2007).

The use of diarrhoea/dag score is by its nature useful at highlighting issues that result in scouring in animals, but do not provide an indication of the cause, e.g., pathogen versus physiological/nutritional. Additionally, diarrhoea is not a clinical sign in all GIN infections and may not be apparent in sub-clinical or early stage infections. The correlation between worm burden and diarrhoea score has been poor in many studies (reviewed by Williams and Palmer, 2012). That said the diarrhoea/dag score has been used in conjunction with other indicators such as FAMACHA, body condition score, sub-mandibular oedema and nasal discharge as part of a Five Point Check© for assessing overall general health in sheep (Bath and Van Wyk, 2009).

The benefits and uses of production parameters such as live weight gain or milk production have been successfully used for targeting anthelmintic administrations (See section below). As a whole, diagnosis of helminth infections combined with decision support tools could be considered of critical importance to counteract the spread of AR by targeting anthelmintic treatments to timings or animal groups at risk and where feasible even supporting individual-level treatment choices. Merging parasite diagnostics with animal performance metrics is attracting renewed research focus in order to reduce the amount of anthelmintics used without jeopardising productive performance (Höglund et al., 2021).

## 3.2 Species specific identification of GIN

As many anthelmintics have a wide spectrum of efficacy against most prevalent GIN species and developmental stages, current anthelmintic treatment practices often make little consideration of the species involved. A changing climate and an associated changing epidemiology as well as an increasing prevalence of AR triggers an increasing need for a better understanding of the GIN species that are present on farm and are causing the largest health and economic impact. The prevalence of AR has also increased at different rates for different species, with large proportions of some species remaining sensitive to treatment for a significantly longer time than others (for example *N. battus* versus *H. contortus*). The ability to make a diagnosis at the species/genus level can enable veterinary practitioners and farmers to make better informed treatment choices. Traditionally, species specific identification requires the culturing of eggs to infective larvae followed by microscopic analysis by a highly trained parasitologist. This takes several days and offers low benefits in terms of actionable diagnostic needs by veterinary practitioners. Recent advances in molecular technologies have made it possible to undertake GIN species identification using various different target sequences (reviewed by Roeber et al., 2013). PCR amplification of the gene of choice, most commonly the internal transcribed spacer (ITS) 2 region, followed by sequencing has made species assessment of eggs, larvae and adult worms possible. ITS is spacer DNA, generally found between the small and large subunits of the ribosomal RNA genes in the chromosome which has low intra-, but high inter-species variability. Deep amplicon sequencing technologies are making it possible to assess both species/genus composition and BZ allele frequency at the population level for both cattle and sheep nematodes (Avramenko et al., 2017, 2019). The approach is a powerful tool in the armoury to help screen large numbers of populations for epidemiological studies (Avramenko et al., 2015) as well as for following the emergence, and/or progression, of AR mutations (Melville et al., 2020).

## 3.3 Digitalisation, biomarkers and precision livestock tools

Electronic identification (EID) of animals has made it possible for farmers to collect, collate and analyse individual animal data at a resolution that was previously difficult to achieve. One area where EID has been extremely useful is in the development of Targeted Selective Treatment (TST) strategies based on live weight gain predictions that rely on the identification of animals that are most detrimentally affected by GIN and therefore likely to respond most positively to treatment (Greer et al., 2009). EID also allows for the analysis and integration of large amounts of data such as pasture and animal conditions, treatment frequencies and dates and animal performance which can assist in on-farm decision making. As faecal egg counting becomes more automated (see below), and therefore quicker to undertake on a large number of animals, FEC data can be more easily used to select animal lines for resistance/resilience to nematode infections, particularly in sheep and goats where there are reasonable correlations between FEC and worm burden for some helminth species (e.g. Cabaret et al., 1998; Coadwell and Ward, 1982; Rinaldi et al., 2009).

The acceptability and uptake of the currently available phenotypic and genotypic tools for GIN assessments are all hampered by one or more of the following issues: time consumption, labour intensity, sensitivity, specificity, precision, accuracy, necessity of specialist equipment and expertise.

As such there is need for pen side and real-time monitoring devices to detect helminth related animal health issues. Previous studies on possible pen-side tests have generally focussed on a single species rather than populations or multiple pathogens. Examples include faecal occult blood detection using dipstick technology (Rodríguez et al., 2015), odour detection using dogs (Richards et al., 2008) or loop-mediated isothermal amplification (LAMP) (Melville et al., 2014) for diagnosis of *H. contortus* infections in sheep. Results from the blood-based marker detection systems (dipstick and odour) suggest they are most effective when the challenge is high but are less useful under modest challenge or in mixed field infections. Table 2 highlights a number of technologies that may have utility for the detection of parasitic diseases, but their further development may require the definition of Target Product Profiles to take into account a fit for purpose application (Entrican et al., 2020).

**Table 2** Novel technologies that may have utility for the detection of helminth infections in livestock ruminants

| Technology | Parasitological examples or potential applications |
|---|---|
| **Individual/animal based** | |
| – Lateral flow-devices, micro-arrays, aptamers based detection systems as well as LAMP | – Sheep scab (lateral flow), *Haemonchus* & liver fluke detection (LAMP) |
| – Sweat, serum or saliva sensors | – CarLA in saliva |
| – Invasive sensors, e.g., biosensors, E-tags, E-pills | – Parasitic gastro-enteritis (PGE)/liver fluke, abomasal/ruminal damage |
| – Non-invasive wearable sensors, e.g., accelerometers, motion detectors, GPS | – PGE, fluke, lungworm |
| – Electronic Identification, automated body condition score, gateway weigh platforms | – General health, decision making PGE |
| – Smell detectors (volatile organic compounds detection) | – Haematophagous infections (e.g. *Haemonchus* spp., fluke) |
| – Biomarkers in meat juices<br>– Biomarkers in faeces | – Tissue/cyst dwelling parasites, GIN infections in beef cattle<br>– Detecting gut inflammation and/or parasite presence (copro-antigen/antibody) |

*Continued*

**Table 2** Novel technologies that may have utility for the detection of helminth infections in livestock ruminants—cont'd

| Technology | Parasitological examples or potential applications |
|---|---|
| – Automated FEC methods based on artificial intelligence/machine learning | – Automated detection and count of GIN eggs, cloud platform for remote diagnosis |
| **Building/infra structure** | |
| – Temperature, infra-red thermography | – General health, oestrus |
| – Sound monitoring | – Coughing housed/grazing cattle; lungworm infection detection |
| – Video monitoring | – General health at housing/lungworm |
| **Environment** | |
| – Drone technology, sensor networks | – Risk mapping (liver fluke, gastro-intestinal nematodes), pasture assessments |
| – Modelling/forecasting | – PGE, liver fluke, lungworm |

Legend. FEC = Feacal Egg Count; GPS = Geo-Positioning System; LAMP = Loop-mediated isothermal amplification; PGE = Parasitic gastro-enteritis; CarLA = Carbohydrate larval antigen.

Digitalised diagnostic methods to detect GIN infection include automated FEC techniques that use artificial intelligence and machine learning for automated recognition and counting of helminth eggs. Research activities in this field have undergone a recent explosion of different methods in development. Described systems, of which some are commercially available, include FECPACKG2, Parasight, Telenostic, VETSCAN IMAGYST, and Kubic FLOTAC Microscope, all based on digital analysis of images taken and computerised counting of parasite eggs (Cain et al., 2020; Cringoli et al., 2021; Elghryani et al., 2020; Nagamori et al., 2020). Validation studies are needed because the diagnostic performance of these new methods have mostly only been published as proof-of-concept results and without precision estimates (Cringoli et al., 2021; Elghryani et al., 2020; Inácio et al., 2020; Li et al., 2019; Lu et al., 2018; Nagamori et al., 2020; Sukas et al., 2019). An exception is the Parasight System that runs on a mobile phone and follows a faecal centrifugation step, exhibiting significantly smaller coefficients of variation when compared with the McMaster method over a range of FEC categories (Slusarewicz et al., 2021).

## 4. Diagnosis of anthelmintic resistance

Integrated parasite control programmes in ruminant livestock will require higher uptake of anthelmintic efficacy and resistance tests. Nevertheless, diagnosis of AR in farm animals has been neglected for years and there has been an historical underinvestment in the development and improvement of diagnostic tools, undermining the secured efficacy of anthelmintic control programmes (Charlier et al., 2018). However, a new impetus by the scientific community and the quickening pace of technological innovations, are promoting a renaissance of interest in developing diagnostic capacity of AR by various international networks and funding organisations dedicated to animal health (Entrican et al., 2020; Morgan et al., 2019).

Different approaches have been proposed and used for the assessment of anthelmintic efficacy and hence for the diagnosis of AR in ruminants, including *in vivo* (faecal egg count reduction test [FECRT] and controlled efficacy test), *in vitro* tests and DNA-based molecular methods (Coles et al., 2006; Taylor et al., 2002). According to a recent stakeholder consultation by the COST Action COMBAR, a combination of *in vivo* (FECRT), *in vitro* and molecular tests was considered needed for assessing the efficacy of anthelmintics and detecting AR (Höglund et al., 2021). The following sections will describe the main characteristics of these different test approaches.

### 4.1 Faecal egg count reduction test

The FECRT is considered the mainstay for the assessment of anthelmintic drug efficacy and hence AR because it is relatively simple and easy-to-perform and has the ability to provide a measure of the performance of a number of different anthelmintics at a time (Kaplan, 2020; Kaplan and Vidyashankar, 2012; McKenna, 2002, 2013). The FECRT is an *in vivo* (or rather *ex vivo*) test based on a simple rationale: the efficacy of an anthelmintic drug is assessed by quantifying the reduction (%) of helminth egg output in a group of animals tested before treatment (Day 0) and between 7 and 21 days after treatment, depending on the drugs used. However, the interpretation of any FECRT is far from simple due to a variety of factors that may affect the outcome. These sources of variation are related to underlying biological processes and the experimental design (pre-analytical phase; e.g., GIN species composition, proportional presence of dose-limiting species, differences in the fecundity among those species, temporary suppression of egg production, physiological status of the animals), the FEC method (analytical phase), and the statistical analysis used for interpretation of the FECRT results (post analytical phase).

The choice of the FEC method (e.g. McMaster, Cornell-Wisconsin, FLOTAC, Mini-FLOTAC, FECPAK) has always represented a trade-off between the costs and ease of use on the one hand and the detection limit and performance parameters (sensitivity, specificity, accuracy and precision) on the other hand (reviewed by Levecke et al., 2012; Nielsen, 2021). Efforts have also been made to more easily interpret the FECRT results and for this purpose specifically designed software packages are freely available, for example the R package "eggCounts" that uses a Bayesian hierarchical model to calculate FECR% and 95% confidence (uncertainty) intervals (Torgerson et al., 2014; Wang et al., 2018). A vision promoted within COMBAR has been that a rapid FECRT based on easy-to-perform, low-cost and reliable tools may increase the user-friendliness and uptake of FECRT by veterinarians, advisors and farmers. Therefore, the following COMBAR criteria have been proposed as a benchmark for an "ideal" FECRT method: Convenient (cheap and easy to use), On-farm use (pen-side and portable), Minimal animal handling and lab requirements (simple and rapid), Biodegradable (eco-friendly), Affordable (for end-users) and Reliable (standardised and repeatable).

There is a widely recognised need for international harmonisation of diagnostic testing for livestock diseases (Holm et al., 2019) and given the multiple sources of variation in the FECRT, this is certainly true for the FECRT to ensure consistent results on anthelmintic efficacy and AR in different settings. The first guidelines for performing a FECRT date back to the early nineties (Coles et al., 1992) by the World Association for the Advancement of Veterinary Parasitology (WAAVP). Over the past 30 years these guidelines have been thoroughly reviewed (Coles et al., 2006) and several studies have provided novel insights regarding the optimal experimental design for the FECRT and the analysis and interpretation of FECRT data (reviewed by Levecke et al., 2018). The latest comprehensive recommendations for performing a FECRT in ruminant livestock have been detailed in Kaplan (2020) and are likely to be consistent with the new WAAVP guidelines which are under development. An important harmonisation effort was also undertaken by the COMBAR consortium, where after a thorough comparison and discussion of experiences and expectations, the participants agreed on simplified FECRT protocols for GIN in cattle, sheep and goats (Annex 1 and 2 in the online version at https://doi.org/10.1016/bs.apar.2021.12.002). These protocols aim to harmonise the procedures from the farm to the lab and the interpretation of the FECRT. They will underpin future field surveys on AR across Europe and allow for better comparison of obtained results between studies and regions.

When applying the FECRT in practice, it should be borne in mind that a multitude of confounders other than AR can cause sub-par FECR%. These include inaccurate anthelmintic administration; variable drug bioavailability including through breed and age differences (e.g. Vercruysse et al., 2008) and diet (e.g. Gaudin et al., 2016); physiological factors including the consequences of GIN infection (e.g. Debackere et al., 1993); and underlying variation in GIN species composition (e.g. McIntyre et al., 2018). Results should be treated with caution when declaring AR on a farm, attention paid to the level of FECR and not just the 95% threshold for classification of efficacy, and the test repeated at intervals to monitor changes in FECR%.

### 4.2 *In vitro* tests

*In vitro* phenotypic tests measure the sensitivity of helminth eggs, larvae or occasionally adult parasites, to drug exposure in laboratory-based tests by detecting the phenotypic effects of anthelmintics on various aspects of helminth-stage development, activity, or viability (reviewed in Demeler et al., 2012b). A list with the *in vitro* tests used for the detection of AR in ruminant GIN field populations is provided in Table 3. The most frequently used tests are: (i) the egg-hatch-inhibition test (EHT) for the evaluation of the effect of BZs on the hatching of ruminant GIN eggs; (ii) the larval development test (LDT) for the evaluation of the susceptibility of sheep GIN to BZs, tetrahydropyrimidines and imidazothiazoles, monepantel and MLs, it was also successfully used to test the effect of MLs and LEV against GINs of cattle (Demeler et al., 2010; Raza et al., 2016) and (iii) the larval migration inhibition test (LMIT) that is used to evaluate GIN susceptibility to MLs (Demeler et al., 2012b).

Compared to the FECRT, these tests all have the advantage of requiring less effort in the field: they require only one faecal sampling and no anthelmintic treatment (Charlier et al., 2018; Kotze et al., 2020). However, among the disadvantages and logistical challenges of the *in vitro* phenotyping tests, is the need to use very fresh faecal material that often must be transported and stored under very specific conditions (for example, to prevent the hatching of GIN eggs prior to the test) and the time (up to 1 week) required for the completion of larval development and thus to obtain the results (Kotze et al., 2020). Furthermore, some *in vitro* tests are limited to a single drug class or for others there appears to be a lack of association between test results and drug

**Table 3** *In vitro* tests for the detection of anthelmintic resistance in livestock ruminants.

| *In vitro* Test | Anthelmintic | Action | References |
|---|---|---|---|
| Egg Hatch Test (EHT) | Benzimidazoles | Inhibition of egg hatching | von Samson-Himmelstjerna et al. (2009); Demeler et al. (2012a) |
| Larval Paralysis and Motility Test (LPAMT)/ Larval Migration Inhibition Test (LMIT) | Macrocyclic lactones<br>Levamisole<br>Morantel | Paralysis of third larval stage | Demeler et al. (2012a)<br>George et al. (2018)<br>Haftu et al. (2020) |
| Larval Development Test (LDT) | Benzimidazoles<br>Levamisole<br>Combinations (benzimidazole/ levamisole)<br>Avermectin<br>Milbemycin | Inhibition of development of third larval stage | Taylor et al. (2002)<br>Raza et al. (2016) |
| Larval Feeding Inhibition Test (LFIT) | Macrocyclic lactones<br>Levamisole | Paralysis of pharyngeal muscles (prevents feeding) of first larval stage | Alvarez-Sánchez et al. (2005a) |
| Adult Development Test (ADT) | Benzimidazoles | Inhibition of development of adult nematode | Small and Coles, 1993; Haftu et al. (2020) |
| Tubulin Binding Test (TBT) | Benzimidazoles | Differential binding of benzimidazoles to tubulin | Taylor et al. (2002) |

susceptibility phenotype. The EHT, for example, only works for BZs (von Samson-Himmelstjerna et al., 2009) and the LMIT has been found to show no differences in ML dose response curves between phenothypically susceptible and resistant populations of *H. contortus* and *Cooperia* spp. (George et al., 2018; Lacey et al., 1995; Ruffell et al., 2018). A recent study indicated that both *in vitro* (EHT and LDT) and molecular testing enabled the detection of low level (approximately 25% resistant alleles) of BZ resistance in *H. contortus* infecting sheep and goats, when the FECRT still showed a FEC reduction of >99% (Königová et al., 2021).

Standard operating procedures of the EHT for the detection of BZ resistance in parasitic nematodes have been successfully developed as a result of a coordinated ring testing in different European countries (von Samson-Himmelstjerna et al., 2009). Other *in vitro* tests (LDT and LMIT) are available to monitor the efficacy of MLs (Demeler et al., 2012a). However, despite their availability, *in vitro* tests are not yet implemented in routine diagnostic laboratory procedures. This may be due to requirements for specific laboratory equipment and technical expertise, the time required to get the results and lack of demand from farmers still not fully convinced of the necessity to test for AR (Charlier et al., 2018). On the other hand, *in vitro* tests are prone to be automated and recently an automated LMIT based on spectrofluorimetric monitoring of the motility of GIN larval stages has been proposed as a decision-making tool to inform anthelmintic treatment decisions in the near future (Charvet et al., 2018).

## 4.3 Molecular tests for AR

Given the limitations of the above-described tests, molecular tests for AR would be a serious advancement to guide appropriate use of anthelmintic drugs. Another advantage of the molecular diagnosis of AR is its superior sensitivity when compared to phenotypic testing (von Samson-Himmelstjerna et al., 2009). To date however, only the molecular detection of BZ resistance provides enough certainty to ensure that the results correspond with the phenotypic status of the examined parasites.

The development of molecular diagnostics for AR is one focus of the Consortium for Anthelmintic Resistance and Susceptibility (CARS), which repeatedly provided overviews on the current status and recent progress of molecular AR testing (Kotze et al., 2014, 2020).

A broad range of drug-target gene associated genetic polymorphisms have been described (for review see von Samson-Himmelstjerna, 2006; Whittaker et al., 2017) with those in tubulin-genes as the BZ target having obtained the largest scientific and experimental attention. Significant associations between drug susceptibility phenotype and resistance-associated allele frequencies were established already decades ago for the beta-tubulin isotype 1 codon 200 TTC/TAC single nucleotide polymorphism (SNP) (von Samson-Himmelstjerna, 2006). However, also other polymorphisms in the beta-tubulin isotype 1 gene, e.g., including SNPs at codons 167 and 198, as well as other genetic changes were demonstrated to contribute to the BZ resistance phenotype in parasitic nematode field isolates. This was particularly shown for *H. contortus* (for review see Kotze and Prichard, 2016) but also demonstrated in other species such as *T. colubriformis* or *T. circumcincta* (Ramünke et al., 2016; Chaudhry et al., 2014, 2015). Accordingly, there are grounds to propose that the testing of ruminant faecal samples can provide meaningful information on the BZ efficacy status. Several methods have been developed to this end (Table 4) including those based on pyrosequencing (von Samson-Himmelstjerna et al., 2009) or more recently on digital droplet PCR-technologies (Baltrušis et al., 2020a) and LAMP (Tuersong et al., 2020). However, the polymorphic genetic nature of BZ-resistant (and likely other drug class resistant) ruminant GIN is a major constraint in the use of methods which are based on the analysis of pre-defined genetic polymorphisms such as those described above. This is demonstrated by the continuing elucidation of additional resistance-associated polymorphisms as for example found in *H. contortus* or *T. circumcincta* field samples at the beta-tubulin codon 198 (Choi et al., 2017; Martínez-Valladares et al., 2020; Mohammedsalih et al., 2020, 2021). Accordingly, it appears to be necessary to always take into consideration that additional/new genetic changes occur in the field and thus any molecular assessment indicating lack of resistance-associated changes must be evaluated with caution.

## 4.4 Omics based approaches for the analysis of AR

With respect to the above outlined limitations associated with the molecular detection of defined resistance-associated genetic changes, the un-directed generation of drug-target or even whole genome sequence information would provide an unbiased insight into the genetic polymorphisms present within the examined pool of DNA-sequences. Recently, next-generation sequencing (NGS) technology based protocols, employing namely the Illumina Miseq platform, have been established for the reliable, accurate and highly sensitive quantitative assessment of BZ resistance associated beta-tubulin alleles in a broad range of ruminant GIN species (Avramenko et al., 2019; Melville et al., 2020; Sargison et al., 2019). Based on the *de-novo* sequencing of approximately 300 bp long beta-tubulin isotype 1 DNA fragments including the three BZ-resistance associated codons this not only allows to assign the obtained sequences to its respective parasite species but also to detect without bias any occurring polymorphism by further sequence comparison. Accordingly, such NGS based approaches provide a considerably more detailed and accurate insight into the BZ resistance situation in the examined worm population as compared to both phenotypic as well as other molecular approaches. NGS based whole genome sequencing has recently

**Table 4** Exemplary list of molecular assays for the assessment of anthelmintic resistance associated genetic polymorphisms in gastrointestinal nematode species of ruminants

| Anthelmintic class | Parasite species | Gene | Allele | Type of method | DNA | References |
|---|---|---|---|---|---|---|
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | F200Y | PCR | Single worm | Roos et al. (1990) |
| Benzimidazole | *Teladorsagia circumcincta* | Beta-tubulin isotype 1 | F200Y | PCR | Single worm | Elard et al. (1999) |
| Benzimidazole | *Haemonchus contortus, Teladorsagia circumcincta* | Beta-tubulin isotype 1 | F167Y | PCR | Single worm | Silvestre and Cabaret, 2002 |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | F200Y | PCR-RFLP | Single worm | Tiwari et al. (2006) |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | F167Y | PCR | Single worm | Ghisi et al. (2007) |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | F200Y | Real-time PCR | Single worm, pools of larvae | Walsh et al. (2007) |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | F167Y, E198A, F200Y | Real-time PCR, pyrosequencing | Pools of larvae | von Samson-Himmelstjerna et al. (2009) |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | F200Y | Digital droplet PCR | Pools of larvae | Baltrušis et al. (2020b) |
| Macrocyclic lactones | *Haemonchus contortus* | Dye-filling protein | A141G and G153T | Digital droplet PCR | Pools of larvae | Elmahalawy et al. (2018) |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | E198A | LAMP | Pools of larvae | Tuersong et al. (2020) |
| Benzimidazole | Numerous trichostrongyles | Beta-tubulin isotype 1 | all | Illumina MiSeq | Pools of larvae | Avramenko et al. (2019) |

*Continued*

**Table 4** Exemplary list of molecular assays for the assessment of anthelmintic resistance associated genetic polymorphisms in gastrointestinal nematode species of ruminants—cont'd

| Anthelmintic class | Parasite species | Gene | Allele | Type of method | DNA | References |
|---|---|---|---|---|---|---|
| Levamisole | *Haemonchus contortus* | Levamisole sensitive acetylcholine receptor (L-AChR1) | Hco-acr-8b exon 3b deletion | qPCR | Pools of eggs | Santos et al. (2019) |
| Benzimidazole | *Haemonchus placei* | Beta-tubulin isotype 1 | F200Y, F167Y | PCR | Single worm | Chaudhry et al. (2014) |
| Benzimidazole | *Cooperia oncophora* | Beta-tubulin isotype 1 | F200 | PCR | Single worm | Njue and Prichard, 2003 |
| Benzimidazole | *Haemonchus contortus* | Beta-tubulin isotype 1 | E198A, F200Y | PCR | Pools of larvae | Ghisi et al. (2007) |
| Benzimidazole | *Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus vitrinus* | Beta-tubulin isotype 1 | F200Y | qPCR | Single worm, pools of larvae | Alvarez-Sánchez et al. (2005b) |
| Benzimidazole | *Trichostrongylus axei* | Beta-tubulin isotype 1 | F200Y | PCR | Single worm | Palcy et al. (2010) |

been employed to study ML resistance associated genomic changes in *H. contortus* (Doyle et al., 2019; Khan et al., 2020). However, both the sequence generation as well as the subsequent bioinformatics analysis require specific instrumentation and expertise which so far are only available in few laboratories.

## 5. Towards a sustainable use of anthelmintics

### 5.1 *Refugia*-based approaches in principle

AR is a heritable trait, and is defined as occurring "when a greater frequency of individuals in a parasite population, usually affected by a dose or concentration of compound, are no longer affected, or a greater concentration of drug is required to reach a certain level of efficacy" (Prichard et al., 1980; Wolstenholme et al., 2004).

The development of AR is an inevitable consequence of the repeated use of anthelmintic drugs, which impose selection pressure on parasite populations and favour the emergence and eventually the dominance of resistant genotypes. Efforts to reduce this selection pressure rely on the preservation of a sub-population of helminths (the so called *refugia*) that are not exposed to anthelmintics (Van Wyk, 2001), and therefore contribute susceptible alleles to the gene pool. The implementation of *refugia*-based strategies depends on numerous factors, including the tolerable parasite burden, mode of inheritance of genes conferring resistance, and degree of mixing of parasite populations (Hodgkinson et al., 2019). There is consequently no universal template for success, and solutions are highly system specific (Greer et al., 2020). Likewise, it is not possible to accurately and objectively estimate the size of *refugia* needed to slow resistance: this will vary according to contextual factors as well as the trade-offs between reduction in anthelmintic coverage and risk of production loss. While models aiming to predict trade-offs between *refugia* generation and production loss exist for livestock systems (e.g. Berk et al., 2016), they operate against a background of uncertainty around the genetic mechanisms of resistance in helminths, and the production impacts of parasites left *in refugia*.

*Refugia* can be generated by avoiding anthelmintic use in animals that are carrying some parasites but performing adequately. This can be at group level, by intervening only when required to support production (targeted treatment; TT), or within a group by identifying individuals that do not require treatment (targeted selective treatment; TST) (Charlier et al., 2014). Delaying treatment at group level can generate a standing crop of susceptible infective larvae to be used continuously, or strategically when needed, to dilute a helminth population that has been more highly selected for resistance. Research attention on how large a population *in refugia* is needed to meaningfully slow AR has so far focused mainly on trade-offs at the animal level, and rarely considers the consequences of livestock movement within farms, and its interactions with climate and weather (Rose et al., 2015), for the fate of eggs released from untreated animals, which generate unevenly distributed *refugia* on pasture. It is rare in modern production systems that livestock mix freely across grazing platforms for the entire grazing season. Some form of grazing management is the norm, and while difficult to incorporate into general models that are transferable across farms, its consequences for the generation and effective utilisation of *refugia* could be profound.

At individual level, *refugia* can be effectively generated by random treatment of individuals (Gaba et al., 2012), but approaches that accurately identify the need for treatment might be more efficient and carry less risk of unintended production loss. As discussed above, indicators for treatment can be broadly divided into parasitological, pathophysiological, or production based parameters (Fig. 1) (Charlier et al., 2014). Individual animal FEC are often impractical or not cost-effective, especially in large herds/flocks, and so indirect indicators are most often considered. The correlations between such indicators and FECs, and hence the *refugia* generated by TST based on them, however, vary widely: for example, weight gain of cattle and sheep in the first grazing season is often quite unrelated to FEC at individual level (e.g. Stafford et al., 2009). The imperfection of indicator-parasite load correlations is actually useful for TST, because a perfect correlation would imply that efficient treatment of ill or underperforming animals tends to generate very limited *refugia* (Fig. 2). Searching for efficient indicators for treatment that reflect parasite burden, therefore, misses the point that inefficient indicators might perform better in slowing AR development, provided health and production are adequately protected.

## 5.2 *Refugia*-based approaches in practice

The fairest answer to the question of "how many animals should be left untreated to generate effective *refugia*", from a theoretical perspective, is therefore "it depends". By the same token, from a practical perspective the answer might be "as many as you can safely get away with". Adverse consequences could arise, however, when applying TT/TST approaches, especially if parasite infection levels are allowed to increase to cause poor

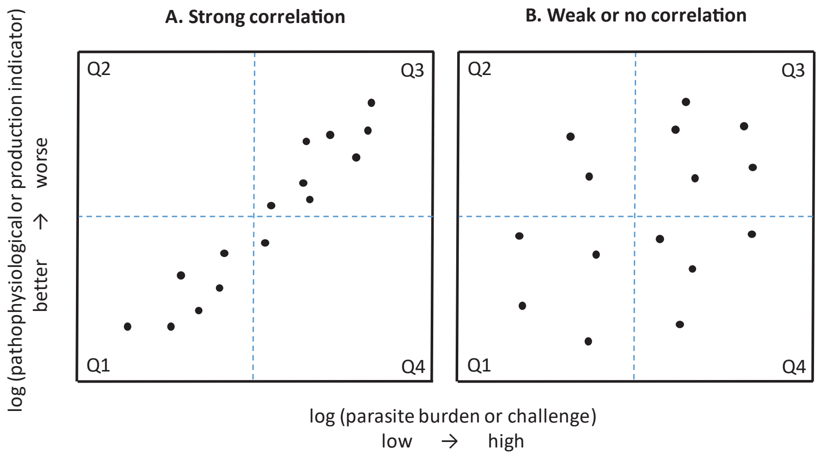


**Fig. 2** Illustration of the relationship between parasite burden (or challenge or faecal egg count) and the detected consequences of infection in terms of ill health or poor performance. In scenario A, the correlation is strong, such that treating under-performing individuals also removes most of the parasites (Q3 = Quadrant 3), and few are left in *refugia* (Q1). In scenario B, the correlation is weak, and treating on the basis of a performance or health threshold leaves some highly infected, but healthy and performing individuals (Q4), which are the largest contributors to *refugia*.

health or production between monitoring points. Confidence in TT and TST is likely to be highest when monitoring of health and production is possible and efficient, at intervals frequent enough to allow timely identification of parasite impacts, and other infections are not facilitated. In cattle, for example, TT/TST can be safely applied in growing animals because impacts of GIN on growth can be quickly detected, as can increases in FEC at group level and diarrhoea; however, lungworm infections can strike with little warning and with costly results (Holzhauer et al., 2011). Many producers in areas afflicted by lungworm (*Dictyocaulus viviparus*) are consequently reluctant to withhold anthelmintic treatment from young cattle in summer, regardless of strong risk mitigation measures for GIN. Even without concurrent loss of production in groups subjected to TT/TST, untreated animals contaminate pastures: while this is necessary to *refugia* generation, it can lead to production losses in animals grazing them later in the season (e.g. Leathwick and Besier, 2014; Höglund et al., 2013).

The availability of equipment for handling and weighing livestock, the time and effort required for monitoring, and the availability of informed professional advice to guide implementation, are also limitations to practical application. Gradually, however, technology is helping to provide solutions, as for example is the form of automated weighing systems, which can be applied to support TST on commercial farms (Stafford et al., 2009) and extended to incorporate growth expectations based on forage availability (Greer et al., 2009). To alleviate the handling and labour requirements of monitoring, sub-groups of lambs can be tracked as sentinels to trigger whole-group interventions or more detailed inspection of individuals for TST (Melville et al., 2021).

Partial automation of TT/TST also has the advantage of alleviating the obligation on farmers to keep sustainable parasite management constantly in mind despite its complexity and the many other demands on their time, thought and energy. Capital investment, however, is often a serious barrier to adoption of equipment such as automated weighing systems. Although the number of studies demonstrating the benefits of TT/TST as *refugia*-based strategies for sustainable parasite control is growing and extending into diverse sectors, there remains uncertainty over how well these will translate to commercial farming systems, and how much they will actually slow AR in them. This must be surmounted before investments in sustainable control are forthcoming from livestock farmers, who often operate on small profit margins. Indicators for TST also exist that do not require such investments, including the FAMACHA system for small ruminants in areas dominated by *H. contortus* (Sargison et al., 2021; Van Wyk and Bath, 2002; Walker et al., 2015). Behavioural monitoring (Hogberg et al., 2021), and novel diagnostic indicators such as those listed in Table 2, also show potential as indicators for treatment in TT/TST systems.

Indicator-led TT/TST can be applied in mature as well as growing livestock, for example on the basis of milk yield and anti-parasite antibody levels. In dairy cows, anti-*O. ostertagi* antibodies in milk are useful to evaluate parasite exposure and potential production losses and to inform control plans and treatment decisions (Charlier et al., 2009, 2010a; Ravinet et al., 2017). Unless accompanied by revised health plans to encourage the build-up of immunity to GIN in replacement stock, however, repeated treatment is likely to be required in many herds, increasing overall anthelmintic use. A holistic approach is needed across the whole production cycle, to increase resilience and reduce reliance on chemical intervention (Charlier et al., 2009; Ravinet et al., 2014).

## 5.3 Global sustainability and parasite control

Societal concerns around chemical use and misuse in food-producing animals, and the environmental impact of farming both locally and in terms of the global greenhouse gas account, are growing and increasingly influencing agricultural policy. It is known that anthelmintics can negatively impact dung-breeding invertebrates such as dung beetles and earthworms (Forbes, 2021), and that these dung fauna are important for incorporation of carbon into grassland soils (Evans et al., 2019). At the same time, greenhouse gas emissions from livestock are likely to be exacerbated if parasite control fails or is voluntarily withdrawn (Fox et al., 2018). Approaches to parasite control that are sustainable in the broadest sense will account for an optimised trade-off not only between production and long-term anthelmintic efficacy, but also between production and environmental outcomes. Maintaining *refugia* for farm biodiversity as well as anthelmintic susceptible helminths will become increasingly important, and can be achieved using targeted worming strategies (Cooke et al., 2017). These strategies are increasingly likely to become required to justify anthelmintic use at all, and in Europe at least occur against a backdrop of advancing regulation of antimicrobial and other veterinary medicines. Chemical treatment is increasingly considered a last resort, and if this is to be realistic for parasite control in livestock, a revolution is needed not only in targeted use of anthelmintics, but also in the effective development and application of non-chemical alternatives to complement them (Vercruysse et al., 2018).

## 6. Prospects of new anthelmintics

Few new anthelmintics are reaching the market. Over the last two decades two new actives have been licensed and launched onto the sheep market in some countries: the amino-acetonitrile derivatives (AAD), i.e., monepantel (Kaminsky et al., 2008), and the spiroindoles (SI), of which derquantel is used in a dual-active product with abamectin (Little et al., 2010). Resistance to these new drugs has been described a few years after their introduction in different GIN species (Bartley et al., 2019; Höglund et al., 2020; Sales and Love, 2016). According to Zajíčková et al. (2020) there are four approaches in the development of new anthelmintics: (i) target-based or high-throughput screening of compound libraries; (ii) the synthesis of new derivatives or new combinations of existing anthelmintics; (iii) the repurposing of currently approved drugs for other therapeutic indications; and (iv) the identification of active plant products, as covered in more detail below. Aside from the option of combination anthelmintics to slow down resistance (Charlier et al., 2018), new anthelmintics should be based on novel modes of action that are well characterised beforehand so that ideally, the anthelmintic use can be combined with a specific test for resistance detection.

Besides the traditional approaches for anthelmintic discovery, several new approaches may be emerging due to blue sky research on novel therapeutics based on, for instance, phage therapy, functional peptides, nanobodies and gene editing approaches. For instance, the 50 Helminth Genomes Project of the Wellcome Sanger Institute may lead to the identification of essential helminth enzymes as novel drug targets. Therefore, understanding the fundamental biology, biochemistry and physiology of parasites can reasonably lead to more effective drug discovery efforts (Vercruysse et al., 2018). However, the development of a completely novel compound is generally very slow and costly, so increasingly efforts are dependent on public-private partnerships between the academic and business sectors. With respect to the strategy called "drug repurposing", its advantage is the availability of preclinical and clinical data that could potentially accelerate the drug development process due to lower costs. Repurposing also offers a great tool for a One Health approach where insights on anthelmintic discovery and efficacy are shared between the human and animal domains in order to advance therapeutic options in both domains (Panic et al., 2014).

## 7. Complementary control approaches

The overall challenge to control GINs in ruminants is to find solutions for the wide variation of situations created by the diversity in host and GIN species, production systems and local epidemiological factors affecting GIN biology. The advent of AR means that we can no longer rely on the use of anthelmintics only as the single option for helminth control. Several other control options (including pasture management, medicinal plants, nematophagous fungi, genetic selection) have long been described and used, but received relatively little attention as the need for their use was low as long as effective chemoprophylactic options were widely available and affordable. Complementary control approaches can reduce the need for anthelmintic use by (i) directly disturbing the worm populations in the gastrointestinal tract, but also by (ii) improving the host response for better resistance or resilience against GINs and (iii) limiting the contact between the host and the infective stages (Torres-Acosta and Hoste, 2008). From a more practical perspective, complementary approaches can also be classified according to the expected time scale to availability. Below we will cover the options that are already available or are expected to be available on the short term (i.e. grazing management, nematophagous fungi and the use of natural bio-active compounds). We do not cover in detail breeding for resistance or control through vaccination, which may be considered as solutions in the long term and for which the reader is referred to reviews by Sutherland and Scott (2010) and Sweeney et al. (2016), and Claerebout and Geldhof (2020), respectively.

## 7.1 Grazing management

Apart from many descriptive studies, describing putative risk factors for increased helminth infection levels related to grazing management (e.g. Springer et al., 2021), few experimental studies have been conducted to underpin effectiveness or feasibility of grazing management options in the last 20 years (Ruiz-Huidobro et al., 2019). This means that current mechanistic insights are still largely based on early work (reviewed by Morley and Donald, 1980 and Stromberg and Averbeck, 1999) or more recently on modelling approaches (Fox et al., 2013; Laurenson et al., 2012).

The general objective of measures of grazing management for GIN control is to reduce the contact between the infective larvae (L3) and the susceptible hosts. This can be achieved by diluting the parasitic risk either in space (e.g. by reducing the stocking rate) or in time (e.g. by exploiting the natural death rate of eggs and larvae on the pastures). However, to act on the pasture systems for parasite control can be very difficult as the primary objective is to provide nutritional resources either by a direct exploitation during the grazing season or by ensuring stored resources (hay, silages). Indeed, when considering specific grazing management practices for parasite control, the economic advantages provided will often not outweigh the disadvantages in terms of labour and resource use (e.g. provision of alternative feeds) (van der Voort et al., 2017). However, it is good veterinary practice to evaluate the applied grazing system on farm and adapt the anthelmintic control strategy accordingly. Correct analysis can identify herds at low risk for excessive helminth infection levels and thus avoid "overprotective" control measures, which seem still common practice on a considerable proportion of herds (Charlier et al., 2010b).

One of the earliest grazing management recommendations for nematode control was the «Dose and Move» concept (Michel, 1969). However, with the increasing emphasis on the *refugia*-based control approaches to tackle AR, this recommendation has currently been widely abandoned. In contrast, other factors such as alternate or simultaneous co-grazing between different hosts, which exploits host specificity by most GIN species to dilute L3 pasture infection levels, move to low-contaminated pasture, rotational grazing, mowing of pastures and reducing grass exposure are still considered applicable elements of an integrated control strategy, which must necessarily be farm-specific.

## 7.2 Nematophagous fungi

Using of nematode-destroying (or nematophagous) microfungi is a preventive approach to control GINs, as it aims at reducing the development of the free-living larval stages and thereby limiting the contact between host and L3. Consequently, reinfection is reduced while no effects are observed on L3 already present in the herbage nor on established infections within the hosts. This is important to keep in mind when these options are implemented.

The concept has been developed over the last 35 years, and recently commercial products were launched. A strain of *Duddingtonia flagrans* (s. *Arthrobotrys flagrans*) (Ascomycetes (Family Orbiliaceae)) was marketed in 2018 as a commercial granulated feed additive "to reduce infective nematode larvae within the manure of grazing animals" in Australia and New Zealand (Bioworma®) (Healey et al., 2018a, 2018b), and a year later *D. flagrans* was included in a similar Brazilian product (Bioverm®) (Braga et al., 2020). Bioworma® is currently also approved in the USA and an application is pending for the EU. Because it is based on the use of one living micro-organism introduced into the environment to obtain control, or reduce effects of a pathogenic target micro-organism, it is defined as biological control. In fact, other micro-organisms could be used for biological control of GINs such as earthworms, dung beetles or mites (Szewc et al., 2021). However, to date nematophagous fungi is the only realistic option in the short term. These microfungi are introduced to the pastures and feed on soil nematodes as well as L1-L3 of GINs, but can also be saprophytic for periods.

In most cases, the application is by feeding resting spores, so-called chlamydospores, to the grazing animals. The spores pass through the gastrointestinal tract and once excreted with faeces, they germinate and proceed with development of hyphae, which form adhesive trapping organs that destroy (or paralyse) larvae within the dung (Grønvold et al., 1993). Oral dosing is currently a necessity to ensure synchronous development of spores to hyphae with that of parasitic eggs to larvae within the dung.

Biocontrol by microfungi offers some advantages to GIN control by commercial drugs. Firstly, it is likely that most GIN species with a direct life cycle and free-living L1-L3 are affected, as clearly evidenced in recent reviews (Canhão-Dias et al., 2020; Szewc et al., 2021). *D. flagrans* has shown reductions in larval counts of 37–98% against the major GIN of sheep, goats and cattle, depending on dose and experimental set-up. If such reductions can be achieved under practical farming conditions, this would be expected to have solid impact on transmission levels. Secondly, it is also most likely that biocontrol is efficacious against drug-resistant GIN strains (Healey et al., 2018b). Further, the approach is non-chemical and therefore without withdrawal period in livestock. There is, however, a caveat as one cannot exclude unwanted substances from the fermentation process remaining in the product (EFSA, 2020). Environmental impact has for years been a much-debated issue in biocontrol in general, e.g., by suppressing indigenous species, but based on existing studies, this has not yet been proven to be a problem for *D. flagrans* (e.g. Faedo et al., 2002; Knox et al., 2002; Paraud et al., 2007a). A limitation to the use of biocontrol, as outlined above, is the need of regular dosing on a daily (or every second/third day) basis lasting in many cases for several weeks or months, which is needed to avoid a build-up of pasture contamination (e.g. Githigia et al., 1997; Nansen et al., 1995). Usually, an even distribution within a group of ruminants can be achieved by combining fungi culture or fungi in sodium alginate pellets with concentrate and ensuring enough trough space per animal to eat.

Worldwide studies within the last two decades, including temperate, subtropical and tropical climates have demonstrated significantly lower pasture larval counts and, in some cases, also reduced FECs after administration of *D. flagrans*, although products and dosing regimes varied greatly (e.g. Assis et al., 2013; de Oliveira et al., 2021; Gómez-Rincón et al., 2006, 2007; Paraud et al., 2007b; Silva et al., 2014; Voinot et al., 2020). However, there still remains a number of field studies, predominantly from temperate regions, showing lack of or insufficient effect of biocontrol in one or more years of trials (e.g. in cattle: Dimander et al., 2003; in sheep: Epe et al., 2009; Eysker et al., 2006; Faessler et al., 2007; in goats: Maingi et al., 2006; Paraud et al., 2007b). The reasons behind the variable effects of biocontrol may relate to the product itself, climate, animals or the composition of pastures and thus diet. Dry conditions are likely to delay the development of both fungi and larvae but may not be the major problem, as long as spores survive. In contrast, heavy rains may result in rapid break-down of dung pats and dispersal of spores away from eggs/larvae and thus, much reduced trapping efficacy. This was the most likely reason for the breakdown of biocontrol in heifers in a Swedish trial (Dimander et al., 2003). Also, the inclusion of (highly) infected animals or pasture with high levels of contamination may result in build-up of pathogenic levels of infections before the biocontrol can be expected to have any diminishing effect (e.g. Eysker et al., 2006; Githigia et al., 1997; Maingi et al., 2006). Interactions with specific types of feed that may result in reduced trapping efficacy cannot be ruled out, e.g., when feeding periparturient ewes on lush spring pastures (Eysker et al., 2006).

Briefly, biocontrol can be a promising and useful alternative to GIN control in several farming systems, e.g., in organic farming systems where there is often plenty of pastures with low levels of contamination available because more grasslands are needed to ensure a proper nitrogen balance. However, the limitations inherent to this principle need to be evaluated. The application requires careful consideration of (1) local climatic conditions, (2) levels of infection in hosts and pasture contamination at the beginning, (3) composition of herd and not least, (4) farmer compliance (frequent dosing). One should prioritise uniform groups at the start, e.g., first season calves or weaned lambs, also to ensure even uptake in all individuals. The use of biocontrol may also preferably be integrated in existing deworming programmes, e.g., in order to remove existing GIN burdens when embarking on biocontrol. In perspective, more fully integrated long-term studies need to be performed while research is in progress to reveal mechanisms behind and to enhance the trapping capacity of nematophagous fungi.

## 7.3 Plant based control

The use of plants to control parasitic helminth infections goes back in history for centuries. It is however with the increasing importance of AR and increasing societal demands to reduce use of chemicals in agriculture that their use has attracted a renewed interest, especially in organic farming. In a modern context, plant based control has been reframed as "nutraceuticals" pointing out that they constitute forages combining nutritional and sanitary properties (Hoste et al., 2015). Whatever the mode of exploitation of plants (i.e. phytotherapeutics *versus* nutraceuticals), the anthelmintic properties have been related to the presence of specific natural compounds described as plant secondary metabolites (PSMs). Herein, we will focus on two models: tannin containing legumes (Fabaceae) and chicory (Astareaceae) containing sesquiterpene lactones.

### 7.3.1 Tannin containing legumes

The early empirical results which suggested that the consumption of bioactive plants might help in GIN control were obtained with a few legume species (sulla, birdfoot and big trefoils) whose peculiarity was to contain condensed tannins (CT) (Niezen et al., 1995, 1998). Since then, further studies in the EU and USA have focused on other CT containing legume fodders, namely sainfoin (*Onobrychis viciifoliae*) and sericea lespedeza (*Lespedeza cuneata*).

The main antiparasitic effects of CT containing legumes have been shown not to be an elimination of the worms in the host, but a combination of effects disturbing biological traits of three key stages of the helminth life cycle: (i) the hatching and development of eggs; (ii) the establishment of L3 in the host and (iii) the adult worms, in particular the fecundity of female worms (Hoste et al., 2015). They have been shown to have significant antiparasitic effects on MDR GINs, thus are an option to consider when AR limits available control options (Gaudin et al., 2016). The anthelmintic properties of these legumes are related to the presence of polyphenols and flavonoids. Two main hypotheses have been evoked to explain the effects of polyphenols on the different GIN stages: (i) a « pharmacological like » (direct) mode of action has been supported in relation with the ability of tannins to bind proteins (Hoste et al., 2012; Williams et al., 2014) and (ii) interactions with the local gut immunity (Andersen-Civil et al., 2021). In addition, a few studies have begun to examine the interactions between the host, the nematode, the presence of tannins and the digestive microbiota (Corrêa et al., 2020).

This basic knowledge, has led to suggest some recommendations for a pertinent use of CT containing plants on farms. Based on the «pharmacological like» hypothesis, it has been proposed that, to be efficient, there is a need to expose GIN in the different digestive organs to a sufficient level of CT and for a sufficient time. In practical terms, this has led to recommend the provision of CT containing resources in feed over a requested threshold of CT in feed for at least 2 weeks (Gaudin et al., 2016).

However, anthelmintic properties of these forages can be highly variable and depend among others on their molecular structure as well as factors affecting their local growth (e.g. climate, stressors and cultivars) (Mueller-Harvey et al., 2018). This severely limits the development of simple recommendations for their use on farms. On the other hand, in support of agroecological approaches, other advantages include their contribution to fix nitrogen in soils and thus, reducing the need for use of fertilisers as well as their potential to reduce greenhouse gas emissions from livestock (Mueller-Harvey et al., 2018).

Thus, a range of modalities to implement CT containing legumes for GIN control have been explored. They include direct grazing as well as the use of conserved forms such as hay (e.g. Shaik et al., 2006), silage or even dehydrated pellets (e.g. Gaudin et al., 2016). Obviously, the conserved forms have the advantages to enable (1) production methods with a high yield of biomass; (2) standardisation, storage and export of the CT resources and (3) measurement of the CT contents supporting their use as nutraceuticals. On the other hand, the exploitation of parcels seeded with CT rich legumes has advantages in terms of autonomous production on farm, and lower economic and environmental ($CO_2$) costs. Whatever the mode of application, their use early in the grazing season seems a priority to delay the dynamics of helminth infections. This timing usually corresponds to the periparturient period in small ruminants and the season when infection pressures are building up. Recently, the use of CT containing agro-industrial waste products is being explored as a new way of GIN control, contributing to the circular economy.

### *7.3.2 Chicory and sesquiterpene lactones*

Most of the abovementioned CT-rich plants with a nutraceutical potential are native to warm climates in Europe. In colder temperate zones a more viable option is chicory (*Cichorium intybus*), a common wild plant used for medicinal purpose for centuries, for human consumption as salad, and 35 years ago developed as a forage crop in New Zealand (Rumball et al., 2003). Chicory is rich in bioactive PSMs, particularly guaianolide sesquiterpene lactones (SL) which are polyphenolic compounds closely related to the former anthelmintic santonin and more distantly related to the anti-malarial drug, artemisinin. SL are most likely implicated in chicory's anthelmintic effect (Foster et al., 2011; Peña-Espinoza et al., 2015), and these studies have confirmed a substantial variation between cultivars with distinct chemical profiles. A recent study has, based on activity guided fractionation and molecular networking, identified 8-deoxylactucin (8-DOL) as one of the main compounds responsible for the *in vitro* anti-parasitic activity (Valente et al., 2021). The study clearly demonstrated that although 8-DOL showed high bioactivity, there was a strong synergy with other isolated compounds showing relatively limited activity on their own. This finding emphasises the concept of nutraceuticals that the beneficial effects most likely stem from the application of the whole or selected parts of plant and not isolated compounds, as compared to pharmaceuticals. However, identification and measurements of responsible compounds will still facilitate the future selection and possibly breeding of the most suitable crops.

Chicory extracts have shown dose-dependent anti-parasitic effect in different *in vitro* tests against a range of important GIN, including *T. circumcincta*, *H. contortus*, *O. ostertagi*, *C. oncophora* and from other clades, *Ascaris suum* (Foster et al., 2011; Peña-Espinoza et al., 2015, 2017; Valente et al., 2021; Williams et al., 2016). A remarkable observation from some of these studies is that larvae exposed to SL-rich extracts become immotile or die without showing signs of structural damage, as often seen after CT exposure. This may imply an anti-parasitic activity related to metabolism, or neuromuscular function of nematodes, as suggested by Peña Espinoza et al. (2018).

Since the original observations of lower FECs and lower *T. circumcincta* burdens in infected lambs grazing pastures with 80% chicory in New Zealand (Scales et al., 1995), several studies with sheep or cattle in New Zealand and Europe, showed marked (up to 65%) reductions in FECs and/or worm counts (recently reviewed by Peña Espinoza et al., 2018). The data suggest that in studies with beneficial effects, the proportion of chicory in the pasture sward or diet has generally been above 60–70% DM of the daily diet while so far, no firm conclusions on critical levels of SL can be drawn. Further, the reductions were mainly in abomasal worm counts while the effect on small intestinal nematode species was low or nil (Peña-Espinoza et al., 2016, 2017). This apparent lack of activity against small intestinal worms in livestock represents a drawback, or at least a limitation for the practical application of chicory depending on prevailing GIN. Similar to CT plants, more knowledge is needed on the fate of SL in the gastrointestinal tract to explain such a lack of efficacy.

In practice, many farmers are reluctant to grow a pure sward of chicory, despite several beneficial agronomical properties, such as a deep taproot and drought resistance. As a high level of intake of chicory and the right timing during the grazing period are needed for optimal effect, the use of preserved feed or industrial by-product becomes an advantage. As an example, extracts from the pulp leftover after extraction of inulin from chicory roots have shown promising anti-parasitic activity (Peña Espinoza et al., 2020), and this approach or other types of bio-refinery deserve further investigation. In brief, at present chicory offers a promising nutraceutical approach to GIN prevention in temperate areas where abomasal parasites prevail.

### *7.3.3 Phytotherapeutics and essential oils*

Besides bioactive plants used as nutraceuticals, there is also a renewed interest in the use and commercialisation of phytotherapeutic drugs and essential oils. Their suspected anthelmintic effects are also associated with the presence of bioactive secondary metabolites. The expansion of these treatments is partly explained by the development of organic farming where plant-based control methods may be favoured over the use of synthetic medicines. However, besides the demonstration of efficacy against the main GIN based on *in vivo* studies (e.g., Mravčáková et al., 2019), one of the main issues is the need to harmonise the status and regulation of phytotherapeutics to address the questions of consumer and environmental safety.

### 7.4 Genetic selection

The selection of livestock to cope with parasite challenge has a long history, especially in sheep, and is not reviewed in detail here. It should be noted, however, that different strategies are possible. Thus, breeding selectively from animals with low FEC is a solid strategy, with moderate heritability and epidemiological benefits through reduced pasture contamination (Sweeney et al., 2016). Sheep bred for parasite resistance can, however, show exacerbated inflammatory responses to GIN infection and reduced individual performance in the face of challenge (Bisset and Morris, 1996). Breeding for good performance under infection pressure, on the other hand—often called resilience—can achieve impressive gains in performance, but potentially at the expense of herd infection levels if this involves immunological tolerance of infection (Morris et al., 2010). In this regard, studies in free-ranging ruminants can be instructive, and show evidence of natural selection for both resistance and tolerance (Hayward et al., 2014). Simultaneously achieving both resistance and resilience through selective breeding in livestock might similarly be possible (Haehling et al., 2021). As reliance on alternatives to anthelmintics increases, the genotypes in which complementary control works best should also arguably be favoured, for example breeds with good vaccine responsiveness (Gonzalez et al., 2019).

## 8. Facilitating behavioural change

### 8.1 Limited adoption of sustainable worm control practices by farmers

Sustainable worm control practices are not routinely applied by sheep farmers (Claerebout et al., 2020; Leathwick and Besier, 2014; McMahon et al., 2013; Moore et al., 2016; Morgan et al., 2012; Morgan and Coles, 2010; Ploeger et al., 2016; Woodgate and Love, 2012), despite the fact that management practices aimed to slow the development of resistance have been advised for some years now (e.g. SCOPS in the UK, www.scops.org.uk). However, the acceptance and implementation of sustainable management practices is better when the recommended practices meet the farmer's criteria for practicality (Leathwick and Besier, 2014). For example, farmers are willing to leave a proportion of the ewes untreated, in order to leave more worms in *refugia* (Leathwick and Besier, 2014; McMahon et al., 2013). Less practical or more labour-intensive practices, such as treating based on FEC, weighing the animals before treatment or testing for AR, are less frequently implemented (Claerebout et al., 2020; Leathwick and Besier, 2014; McMahon et al., 2013; Ploeger et al., 2016). Among cattle farmers, convenience seems even more important for uptake than for sheep or mixed farmers (Easton et al., 2018). In general, cattle farmers seem to be less compelled to adopt sustainable worm control practices (Leathwick and Besier, 2014; McArthur and Reinemeyer, 2014; USDA, 2010), although most organic cattle farmers in Europe express willingness to invest labour and cost in alternative practices (Takeuchi-Storm et al., 2021).

Improving uptake of sustainable worm management practices will require an uncomplicated and understandable message about resistance, and recommendations that are practical as well as effective. Collaboration among stakeholders will be essential, but to influence farmers and their advisors, we need to be conscious of how individuals and groups change their minds (McArthur and Reinemeyer, 2014).

## 8.2 Barriers and incentives for farmers to adopt sustainable worm control practices

Although practicality and cost-effectiveness of parasite control measures are important to farmers, their decisions are not based on rational arguments alone, but are influenced by their personal traits and social environment. Therefore, socio-psychological theories, e.g., the *Theory of Planned Behaviour* (Ajzen, 1991) and the *Health Belief Model* (Rosenstock et al., 1988) and related methodologies have been used to explore farmers' intention to adopt sustainable worm control practices.

Knowledge and awareness of AR were identified as important factors for the uptake of sustainable control practices by UK sheep farmers (Jack et al., 2017). This is in agreement with an earlier survey that identified low awareness of both the risk of AR and concomitant information campaigns as barriers for the adoption of sustainable practices (Morgan et al., 2012). Low infection awareness and low priority of the disease were also identified as important barriers for dairy farmers' positive intentions towards sustainable GIN control (Vande Velde et al., 2018a). However, AR was not perceived as a risk, and had no effect on the adoption intentions of diagnostic methods for worm control in dairy farmers (Vande Velde et al., 2015), which may be explained by the slower development of AR in cattle compared to sheep (Coles, 2002). A positive attitude towards (their current use of) anthelmintics is a barrier for possible uptake of sustainable practices in both sheep and dairy cattle farmers (Morgan et al., 2012; Vande Velde et al., 2015), while a positive attitude towards diagnostics and the perceived pressure of the subjective norms were the main drivers of this intention in dairy farmers (Vande Velde et al., 2015). These subjective norms were identified as the opinion of the veterinarian and of their fellow farmers (Vande Velde et al., 2018a). However, farmers hold a contrasting relationship with both norms throughout the different stages of behaviour: although they do not value other farmers' opinions as a positive reference in the behaviour intention phase, they do follow and mimic their peers' behaviour as a group in the action phase, illustrating a gap between behaviour intention and actual behaviour. Similarly, the veterinarian was identified as the most important positive reference for the farmers' adoption intention, but also as the responsible actor for the actual application of worm control. As such, the farmers did not hold themselves responsible for implementing sustainable worm control strategies (Vande Velde et al., 2018a). Finally, planning was suggested as an important factor to perform and maintain adopted sustainable practices, which could help to surmount other suggested barriers for actual adoption, i.e., habits and responsibility (Vande Velde et al., 2018a).

## 8.3 The role of the veterinarian

Other studies also indicate an important role for the veterinarian to improve uptake of sustainable worm control practices by farmers. The awareness of the TST approach in sheep farmers was greatest where professional advisers were consulted regarding worm control (Cornelius et al., 2015) and UK farmers who bought anthelmintics from veterinarians were more likely to be informed about diagnostic-led control advice (Easton et al., 2018). (Cattle) veterinarians recognise their influence and the need to be proactive advisors, but struggle with acting upon this awareness to inspire farmer behaviour change (Bard et al., 2019). To improve farmers' adherence with veterinary advice, a farmer-centred approach is recommended, based on trust and focusing on the farmer's needs and priorities (Bard et al., 2019; Svensson et al., 2019). Farmers' satisfaction following farm visits by the veterinarian increased with their perception of farmer-centeredness score

(DeGroot et al., 2021) and farmers' preparedness to adopt veterinary advice is positively associated with their satisfaction (Ritter et al., 2019). According to Svensson et al. (2019), the most commonly stated reasons for adherence to the veterinarian's advice related to trust (in the veterinarian, in the advisory process, or in individual preventive measures). The most common reasons not to follow the recommended advice were related to feasibility.

Obviously, a requirement for veterinarians to advise sustainable worm control practices to their clients, is their ability and willingness to promote sustainable worm control. Historically, the management advice of practitioners has not relied strongly on parasite epidemiology, and practitioners may not have the knowledge to implement evidence-based recommendations (McArthur and Reinemeyer, 2014). However, a more recent study investigating UK anthelmintic prescribers indicated a good knowledge of basic helminthology and best practice guidelines among livestock veterinarians (Easton et al., 2016). Surprisingly, despite a central role of veterinarians in communicating sustainable worm control practices, no data are available on factors influencing the veterinarian's adoption (intention) of sustainable worm control. A study on antibiotic prescription practices among veterinarians in the USA indicated that the odds of compliance with prescription policies were 4-times higher among veterinarians working at facilities that had prescription policies compared to those at facilities that didn't. The authors recommended that more veterinary practices should be encouraged to adopt prescription policies to help improve compliance and reduce antimicrobial resistance (Odoi et al., 2021). Similarly, sustainable worm control guidelines may help to avoid that contradictory advice is given to livestock owners by veterinarians (Kenyon et al., 2017).

## 8.4 Further research towards practical application

Clearly, more research is needed to identify potential incentives and barriers for farmers and veterinarians to adopt sustainable worm control practices. Data obtained from descriptive assessments or socio-psychological behaviour models should be validated experimentally (e.g. Vande Velde et al., 2017) before they are used in communication strategies. Moreover, there are often other factors influencing farmers' decisions that are not (or less) internally driven, such as the farmer's socio-economic and regulatory environment (Charlier et al., 2016) and, since farmers' and veterinarians' behaviour is just regular human behaviour, the majority of their decisions are based on intuition and unconscious paths. These additional drivers should be taken into consideration to obtain a profound view of the farmers' and veterinarians' behaviour (Vande Velde et al., 2018b).

Historically, information transfer has occurred in a unidirectional fashion, rather than as an exchange of views by all interested parties (Kenyon et al., 2017). Instead of this top-down approach, Wilson et al. (2015) suggested a systems approach where knowledge is built and shared through equal involvement of different stakeholders, which would meet the farmers' expectations of a more farmer-centred communication style. Considering behaviour is not always consciously driven, other methods, focusing on unconscious paths (or heuristics) can also be promising tools for future campaigns. A mixture of both unconscious (e.g. nudging) and conscious methods (e.g. processing and generating information) may eventually have the best effect on changing farmers' behaviour in the long run (Garza et al., 2020; Vande Velde et al., 2018b).

## 9. Conclusions

By way of their inherent genetic diversity, GIN parasites infecting ruminant livestock have consistently found ways to circumvent human imposed control measures. On top of an altering epidemiology due to climate change and associated farm husbandry practices, we are faced with an escalating spread of AR. Although the overall economic impact of AR on a population level is still limited, it is already negatively impacting on the profitability of individual farms and is becoming a serious hurdle to maintain and promote pasture-based ruminant production systems. To equip the veterinary toolbox and keep pace with the genetically evolving parasites, research pipelines for improved diagnostics, vaccines, new therapeutics & improved therapeutics responses as well as complementary control approaches should be expanded. This will take time, thus, on the short term there is need for learning to use the current tools sustainably, preserve the efficacy of current anthelmintics and minimise bystander effects of toxic residues in the environment. This should contribute to reduce the important burden which nematodes continue to cause in terms of animal health, welfare, economic and environmental impact while, in the meantime, tackling AR. In order to support these activities, COMBAR needs to evolve from a science coordination network into a transnational, multi-actor initiative that develops indicators of infection and sustainable control, sets targets and promotes good practices to achieve them. Recommendations should be developed at various levels (world, region, country, production system, farm) to provide a rational guidance on the use of anthelmintics, thus requiring local solutions for the global challenge of AR. Such approaches have already shown success in other fields of the antimicrobial resistance conundrum (i.e. antibiotics), but will require a new intensity and more structural collaboration in the anthelmintic field than what exists today. If both researchers, animal medicines industry, veterinarians, advisors and farmers contribute within their merits and responsibility, supported through international funding mechanism, AR can become a problem of the past.

## Acknowledgements

This article is based upon work from the COST Action COMBAR CA16230, supported by COST (European Cooperation in Science and Technology). SMT received funding by The Danish Council for Independent Research (Grant DFF–6111-00394). DJB received funding from the Scottish Government: Rural & Environment Science & Analytical Services. ERM received funding from UK Research and Innovation through grant BB/R010250/1.

## References

Ajzen, I., 1991. The theory of planned behavior. Organ. Behav. Hum. Decis. Process. 50, 179–211.

Alvarez-Sánchez, M.A., Pérez García, J., Bartley, D., Jackson, F., Rojo-Vázquez, F.A., 2005a. The larval feeding inhibition assay for the diagnosis of nematode anthelmintic resistance. Exp. Parasitol. 110, 56–61.

Alvarez-Sánchez, M.A., Pérez-García, J., Cruz-Rojo, M.A., Rojo-Vázquez, F.A., 2005b. Real time PCR for the diagnosis of benzimidazole resistance in trichostrongylids of sheep. Vet. Parasitol. 129, 291–298.

Andersen-Civil, A.L.S., Pankaj, A., Williams, A.R., 2021. Regulation of enteric infection and immunity by dietary proanthocyanidins. Front. Immunol. 12, 637603.

Assis, R.C.L., Luns, F.D., Araújo, J.V., Braga, F.R., Assis, R.L., Marcelino, J.L., Freitas, P.C., Andrade, M.A.S., 2013. Comparison between the action of nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* in the biological control of bovine gastrointestinal nematodiasis in tropical southeastern Brazil. Vet. Parasitol. 193, 134–140.

Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J.S., 2015. Exploring the gastrointestinal "nemabiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS One 10, e0143559.

Avramenko, R.W., Redman, E.M., Lewis, R., Bichuette, M.A., Palmeira, B.M., Yazwinski, T.A., Gilleard, J.S., 2017. The use of nemabiome metabarcoding to explore gastro-intestinal nematode species diversity and anthelmintic treatment effectiveness in beef calves. Int. J. Parasitol. 47, 893–902.

Avramenko, R.W., Redman, E.M., Melville, L., Bartley, Y., Wit, J., Queiroz, C., Bartley, D.J., Gilleard, J.S., 2019. Deep amplicon sequencing as a powerful new tool to screen for sequence polymorphisms associated with anthelmintic resistance in parasitic nematode populations. Int. J. Parasitol. 49, 13–26.

Baltrušis, P., Halvarsson, P., Höglund, J., 2020a. Utilization of droplet digital PCR to survey resistance associated polymorphisms in the β tubulin gene of *Haemonchus contortus* in sheep flocks in Sweden. Vet. Parasitol. 288, 109278.

Baltrušis, P., Komáromyová, M., Várady, M., von Samson-Himmelstjerna, G., Höglund, J., 2020b. Assessment of the F200Y mutation frequency in the β tubulin gene of *Haemonchus contortus* following the exposure to a discriminating concentration of thiabendazole in the egg hatch test. Exp. Parasitol. 217, 107957.

Bard, A.M., Main, D., Roe, E., Haase, A., Whay, H.R., Reyher, K.K., 2019. To change or not to change? Veterinarian and farmer perceptions of relational factors influencing the enactment of veterinary advice on dairy farms in the United Kingdom. J. Dairy Sci. 102, 10379–10394.

Bartley, D.J., Skuce, P.J., Zadoks, R.N., MacLeod, M., 2016. Endemic sheep and cattle diseases and greenhouse gas emissions. Adv. Anim. Biosc. 7, 253–255.

Bartley, D.J., Hamer, K., Andrews, L., Sargison, N.D., Morrison, A.A., 2019. Multigeneric resistance to monepantel on a UK sheep farm. Vet. Parasitol. 276S, 100003.

Bartley, D.J., Jewell, N.J., Andrews, L.M., Mitchell, S., Morrison, A.A., 2021. Molecular and phenotypic characterisation of fenbendazole resistance in a field-derived isolate of *Ostertagia ostertagi*. Vet. Parasitol. 289, 109319.

Bath, G.F., Van Wyk, J.A., 2009. The five point check© for targeted selective treatment of internal parasites in small ruminants. Small. Rum. Res. 86, 6–13.

Berk, Z., Laurenson, Y.C.S.M., Forbes, A.B., Kyriazakis, I., 2016. Modelling the consequences of targeted selective treatment strategies on performance and emergence of anthelmintic resistance amongst grazing calves. Int. J. Parasitol. Drugs Drug Resist. 6, 258–271.

Bisset, S.A., Morris, C.A., 1996. Feasibility and implications of breeding sheep for resilience to nematode challenge. Int. J. Parasitol. 26, 857–868.

Blake, N., Coles, G., 2007. Flock cull due to anthelmintic-resistant nematodes. Vet. Rec. 161, 36.

Borges, F.A., Almeida, G.D., Heckler, R.P., Lemes, R.T., Onizuka, M.K., Borges, D.G., 2013. Anthelmintic resistance impact on tropical beef cattle productivity: effect on weight gain of weaned calves. Tropl. Anim. Health Prod. 45, 723–727.

Boyce, W., Kollias, G., Courtney, C.H., Allen, J., Chalmers, E., 1984. Efficacy of ivermectin against gastrointestinal nematodes in dromedary camels. J. Am. Vet. Med. Assoc. 185, 1307–1308.

Boyce, C., Jaye, C., Noller, G., Bryan, M., Doolan-Noble, F., 2021. *Mycoplasma bovis* in New Zealand: a content analysis of media reporting. Kōtuitui. New Z. J. Soc. Sci. Online, 1–21.

Braga, F.R., Ferraz, C.M., da Silva, E.N., de Araújo, J.V., 2020. Efficiency of the Bioverm (*Duddingtonia flagrans*) fungal formulation to control *in vivo* and *in vitro* of *Haemonchus contortus* and *Strongyloides papillosus* in sheep. 3. Biotech 10, 1–5.

Cabaret, J., Gasnier, N., Jacquiet, P., 1998. Faecal egg counts are representative of digestive-tract strongyle worm burdens in sheep and goats. Parasite 5, 137–142.

Cain, J.L., Slusarewicz, P., Rutledge, M.H., McVey, M.R., Wielgus, K.M., Zynda, H.M., Wehling, L.M., Scare, J.A., Steuer, A.E., Nielsen, M.K., 2020. Diagnostic performance of McMaster, Wisconsin, and automated egg counting techniques for enumeration of equine strongyle eggs in fecal samples. Vet. Parasitol. 284, 109199.

Candy, P.M., Waghorn, T.S., Miller, C.M., Ganesh, S., Leathwick, D.M., 2018. The effect on liveweight gain of using anthelmintics with incomplete efficacy against resistant *Cooperia oncophora* in cattle. Vet. Parasitol. 251, 56–62.

Canhão-Dias, M., Paz-Silva, A., Madeira de Carvalho, L.M., 2020. The efficacy of predatory fungi on the control of gastrointestinal parasites in domestic and wild animals—a systematic review. Vet. Parasitol. 283, 109173.

Charlier, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P., Vercruysse, J., 2009. Gastrointestinal nematode infections in adult dairy cattle: impact on production, diagnosis and control. Vet. Parasitol. 164, 70–79.

Charlier, J., Vercruysse, J., Smith, J., Vanderstichel, R., Stryhn, H., Claerebout, E., Dohoo, I., 2010a. Evaluation of anti-*Ostertagia* antibodies in individual milk samples as decision parameter for selective anthelmintic treatment in dairy cows. Prev. Vet. Med. 93, 147–152.

Charlier, J., Demeler, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P., Vercruysse, J., 2010b. *Ostertagia ostertagi* in first-season grazing cattle in Belgium, Germany and Sweden: general levels of infection and related management practices. Vet. Parasitol. 171, 91–98.

Charlier, J., Dorny, P., Levecke, B., Demeler, J., von Samson-Himmelstjerna, G., Höglund, J., Vercruysse, J., 2011. Serum pepsinogen levels to monitor gastrointestinal nematode infections in cattle revisited. Res. Vet. Sci. 90, 451–456.

Charlier, J., Morgan, E.R., Rinaldi, L., van Dijk, J., Demeler, J., Höglund, J., Hertzberg, H., Van Ranst, B., Hendrickx, G., Vercruysse, J., Kenyon, F., 2014. Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. Vet. Rec. 175, 250–255.

Charlier, J., De Waele, V., Ducheyne, E., van der Voort, M., Vande Velde, F., Claerebout, E., 2016. Decision making on helminths in cattle: diagnostics, economics and human behaviour. Ir. Vet. J. 69, 14.

Charlier, J., Thamsborg, S.M., Bartley, D.J., Skuce, P.J., Kenyon, F., Geurden, T., Hoste, H., Williams, A.R., Sotiraki, S., Höglund, J., Chartier, C., Geldhof, P., van Dijk, J., Rinaldi, L., Morgan, E.R., von Samson-Himmelstjerna, G., Vercruysse, J., Claerebout, E., 2018. Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs. Transbound. Emerg. Dis. 65, 217–234.

Charlier, J., Rinaldi, L., Musella, V., Ploeger, H.W., Chartier, C., Vineer, H.R., Hinney, B., von Samson-Himmelstjerna, G., Băcescu, B., Mickiewicz, M., Mateus, T.L., Martinez-Valladares, M., Quealy, S., Azaizeh, H., Sekovska, B., Akkari, H., Petkevicius, S., Hektoen, L., Höglund, J., Morgan, E.R., Bartley, D.J., Claerebout, E., 2020. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. Prev. Vet. Med. 182, 105103.

Charvet, C.L., Guégnard, F., Courtot, E., Cortet, J., Neveu, C., 2018. Nicotine-sensitive acetylcholine receptors are relevant pharmacological targets for the control of multidrug resistant parasitic nematodes. Int. J. Parasitol. Drugs Drug Resist. 8, 540–549.

Chaudhry, U., Miller, M., Yazwinski, T., Kaplan, R., Gilleard, J., 2014. The presence of benzimidazole resistance mutations in *Haemonchus placei* from US cattle. Vet. Parasitol. 204, 411–415.

Chaudhry, U., Redman, E.M., Raman, M., Gilleard, J.S., 2015. Genetic evidence for the spread of a benzimidazole resistance mutation across southern India from a single origin in the parasitic nematode *Haemonchus contortus*. Int. J. Parasitol. 45, 721–728.

Choi, Y.J., Bisset, S.A., Doyle, S.R., Hallsworth-Pepin, K., Martin, J., Grant, W.N., Mitreva, M., 2017. Genomic introgression mapping of field-derived multiple-anthelmintic resistance in *Teladorsagia circumcincta*. PLoS Genet. 13, e1006857.

Claerebout, E., Geldhof, P., 2020. Helminth vaccines in ruminants: from development to application. Vet. Clin. North Am. Food Anim. Pract. 36, 159–171.

Claerebout, E., De Wilde, N., Van Mael, E., Casaert, S., Vande Velde, F., Roeber, F., Vinueza Veloz, P., Levecke, B., Geldhof, P., 2020. Anthelmintic resistance and common worm control practices in sheep farms in Flanders, Belgium. Belgium. Vet. Parasitol. Reg. Stud. Reports. 20, 100393.

Coadwell, W.J., Ward, P.F., 1982. The use of faecal egg counts for estimating worm burdens in sheep infected with *Haemonchus contortus*. Parasitology 85, 251–256.

Coles, G.C., 2002. Cattle nematodes resistant to anthelmintics: why so few cases? Vet. Res. 33, 481–489.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of vet. Parasitol. (W.a.a.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136, 167–185.

Conder, G.A., Campbell, W.C., 1995. Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. Adv. Parasitol. 35, 1–84.

Cooke, A.S., Morgan, E.R., Dungait, J.A.J., 2017. Modelling the impact of targeted anthelmintic treatment of cattle on dung fauna. Environ. Toxicol. Pharmacol. 55, 94–98.

Cornelius, M.P., Jacobson, C., Besier, R.B., 2015. Factors likely to influence the adoption of targeted selective treatment strategies by sheep farmers in Western Australia. Prev. Vet. Med. 121, 325–331.

Corrêa, P.S., Mendes, L.W., Lemos, L.N., Crouzoulon, P., Niderkorn, V., Hoste, H., Costa-Júnior, L.M., Tsai, S.M., Faciola, A.P., Abdalla, A.L., Louvandini, H., 2020. Tannin supplementation modulates the composition and function of ruminal microbiome in lambs infected with gastrointestinal nematodes. FEMS Microb. Ecol. 96 (fiaa024).

Crimes, D., Enticott, G., 2019. Assessing the social and psychological impacts of endemic animal disease amongst farmers. Front. Vet. Sci. 6, 342.

Cringoli, G., Amadesi, A., Maurelli, M.P., Celano, B., Piantadosi, G., Bosco, A., Ciuca, L., Cesarelli, M., Bifulco, P., Montresor, A., Rinaldi, L., 2021. The Kubic FLOTAC microscope (KFM): a new compact digital microscope for helminth egg counts. Parasitology 14, 427–434.

de Oliveira, L.S.S.C.B., Dias, F.G.S., Melo, A.L.T., de Carvalho, L.M., Silva, E.N., Araújo, J.V.D., 2021. Bioverm® in the control of nematodes in beef cattle raised in the central-west region of Brazil. Pathogens. 10, 548.

Debackere, M., Landuyt, J., Vercruysse, J., McKellar, Q., 1993. The influence of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* infections on the pharmacokinetics of febantel in lambs. J. Vet. Pharmacol. Therap. 16, 261–274.

DeGroot, A., Coe, J., Kelton, D., Miltenburg, C., Wichtel, J., Duffield, T., 2021. Factors associated with food-animal producer visit-specific satisfaction following on-farm interaction with a veterinarian. Vet. Rec. 188, e15.

Demeler, J., Küttler, U., von Samson-Himmelstjerna, G., 2010. Adaptation and evaluation of three different *in vitro* tests for the detection of resistance to anthelmintics in gastrointestinal nematodes of cattle. Vet. Parasitol. 170, 61–70.

Demeler, J., Kleinschmidt, N., Küttler, U., Koopmann, R., von Samson-Himmelstjerna, G., 2012a. Evaluation of the egg hatch assay and the larval migration inhibition assay to detect anthelmintic resistance in cattle parasitic nematodes on farms. Parasitol. Int. 61, 614–618.

Demeler, J., Schein, E., von Samson-Himmelstjerna, G., 2012b. Advances in laboratory diagnosis of parasitic infections of sheep. Vet. Parasitol. 189, 52–64.

Dimander, S.O., Höglund, J., Uggla, A., Spörndly, E., Waller, P.J., 2003. Evaluation of gastro-intestinal nematode parasite control strategies for first-season grazing cattle in Sweden. Vet. Parasitol. 111, 193–209.

Dorny, P., Stoliaroff, V., Charlier, J., Meas, S., Sorn, S., Chea, B., Holl, D., Van Aken, D., Vercruysse, J., 2011. Infections with gastrointestinal nematodes, *Fasciola* and *Paramphistomum* in cattle in Cambodia and their association with morbidity parameters. Vet. Parasitol. 175, 293–299.

Doyle, S.R., Illingworth, C., Laing, R., Bartley, D.J., Redman, E., Martinelli, A., Holroyd, N., Morrison, A.A., Rezansoff, A., Tracey, A., Devaney, E., Berriman, M., Sargison, N., Cotton, J.A., Gilleard, J.S., 2019. Population genomic and evolutionary modelling analyses reveal a single major QTL for ivermectin drug resistance in the pathogenic nematode, *Haemonchus contortus*. BMC Genomics 20, 218.

Easton, S., Bartley, D.J., Hotchkiss, E., Hodgkinson, J.E., Pinchbeck, G.L., Matthews, J.B., 2016. Use of a multiple choice questionnaire to assess UK prescribing channels' knowledge of helminthology and best practice surrounding anthelmintic use in livestock and horses. Prev. Vet. Med. 128, 70–77.

Easton, S., Pinchbeck, G.L., Bartley, D.J., Hodgkinson, J.E., Matthews, J.B., 2018. A survey of experiences of UK cattle and sheep farmers with anthelmintic prescribers; are best practice principles being deployed at farm level? Prev. Vet. Med. 155, 27–37.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2020. Scientific opinion on the safety and efficacy of BioWorma® (*Duddingtonia flagrans* NCIMB 30336) as a feed additive for all grazing animals. EFSA J. 18, 6208.

El-Abdellati, A., Geldhof, P., Claerebout, E., Vercruysse, J., Charlier, J., 2010. Monitoring macrocyclic lactone resistance in *Cooperica oncophora* on a Belgian cattle farm during four consecutive years. Vet. Parasitol. 171, 167–171.

Elard, L., Cabaret, J., Humbert, J.F., 1999. PCR diagnosis of benzimidazole-susceptibility or -resistance in natural populations of the small ruminant parasite, Teladorsagia circumcincta. Vet. Parasitol. 80, 231–237.

Elghryani, N., Crispell, J., Ebrahimi, R., Krivoruchko, M., Lobaskin, V., McOwan, T., O'Connor, W., Power, E., Voisin, B., Scholz, D., de Waal, T., 2020. Preliminary evaluation of a novel, fully automated, Telenostic device for rapid field-diagnosis of cattle parasites. Parasitology 147, 1249–1253.

Elmahalawy, S.T., Halvarsson, P., Skarin, M., Höglund, J., 2018. Genetic variants in dyf-7 validated by droplet digital PCR are not drivers for ivermectin resistance in *Haemonchus contortus*. Int. J. Parasitol. Drugs Drug Resist. 8, 278–286.

Entrican, G., Charlier, J., Dalton, L., Messori, S., Sharma, S., Taylor, R., Morrow, A., 2020. Construction of generic roadmaps for the strategic coordination of global research into infectious diseases of animals and zoonoses. Transbound. Emerg. Dis. 68, 1513–1520.

Epe, C., Holst, C., Koopmann, R., Schnieder, T., Larsen, M., von Samson-Himmelstjerna, G., 2009. Experiences with *Duddingtonia flagrans* administration to parasitized small ruminants. Vet. Parasitol. 159, 86–90.

Evans, K.S., Mamo, M., Wingeyer, A., Schacht, W.H., Eskridge, K.M., Bradshaw, J., Ginting, D., 2019. Soil fauna accelerate dung pat decomposition and nutrient cycling into grassland soil. Rangeland Ecol. Manag. 72, 667–677.

Eysker, M., Bakker, N., Kooyman, F.N.J., Olthuis, S.O., Ploeger, H.W., 2006. Effect of biological control through the daily application of spores of *Duddingtonia flagrans* in lambs kept under an evasive grazing system in the Netherlands. Vet. Parasitol. 140, 312–320.

Ezenwa, V.O., Civitello, D.J., Barton, B.T., Becker, D.J., Brenn-White, M., Classen, A.T., Deem, S.L., Johnson, Z.E., Kutz, S., Malishev, M., Penczykowski, R.M., Preston, D.L., Vannatta, J.T., Koltz, A.M., 2020. Infectious diseases, livestock, and climate: a vicious cycle? Trends Ecol. Evol. 35, 959–962.

Faedo, M., Larsen, M., Dimander, S.O., Yeates, G.W., Höglund, J., Waller, P.J., 2002. Growth of the fungus *Duddingtonia flagrans* in soil surrounding feces deposited by cattle or sheep fed the fungus to control nematode parasites. Biol. Cont. 23, 64–70.

Faessler, H., Torgerson, P.R., Hertzberg, H., 2007. Failure of *Duddingtonia flagrans* to reduce gastrointestinal nematode infections in dairy ewes. Vet. Parasitol. 147, 96–102.

Fairweather, I., Brennan, G.P., Hanna, R.E.B., Robinson, M.W., Skuce, P.J., 2020. Drug resistance in liver flukes. Int. J. Parasitol. Drugs Drug Resist. 12, 39–59.

Fazzio, L.E., Sánchez, R.O., Streitenberger, N., Galvan, W.R., Giudici, C.J., Gimeno, E.J., 2014. The effect of anthelmintic resistance on the productivity in feedlot cattle. Vet. Parasitol. 206, 240–245.

Forbes, A.B., 2021. Environmental risk assessment of veterinary parasiticides used in cattle. Livestock. 26, 15–25.

Forbes, A.B., Warren, M., Upjohn, M., Jones, J., Charlier, J., Fox, M.T., 2009. Associations between blood gastrin, ghrelin, leptin, pepsinogen and *Ostertagia ostertagi* antibody concentrations and voluntary feed intake in calves exposed to a trickle infection with *O. ostertagi*. Vet. Parasitol. 162, 295–305.

Foster, J.G., Joyce, G., Cassida, K.A., Turner, K.E., 2011. *In vitro* analysis of the anthelmintic activity of forage chicory (*Cichorium intybus L.)* sesquiterpene lactones against a predominantly *Haemonchus contortus* egg populations. Vet. Parasitol. 180, 296–306.

Fox, N.J., Marion, G., Davidson, R.S., White, P.C., Hutchings, M.R., 2013. Modelling parasite transmission in a grazing system: the importance of host behaviour and immunity. PLoS One 8, e77996.

Fox, N.J., Smith, L.A., Houdijk, J.G.M., Athanasiadou, S., Hutchings, M.R., 2018. Ubiquitous parasites drive a 33% increase in methane yield from livestock. Int. J. Parasitol. 48, 1017–1021.

Gaba, S., Cabaret, J., Chylinski, C., Sauve, C., Cortet, J., Silvestre, A., 2012. Can efficient management of sheep gastro-intestinal nematodes be based on random treatment? Vet. Parasitol. 190, 178–184.

Garza, M., Agren, C.C., Lindberg, A., 2020. Nudging in animal disease control and surveillance: a qualitative approach to identify strategies used to improve compliance with animal health policies. Front. Vet. Sci. 7, 383.

Gasbarre, L.C., Leighton, E.A., Bryant, D., 1996. Reliability of a single fecal egg per gram determination as a measure of individual and herd values for trichostrongyle nematodes of cattle. Am. J. Vet. Res. 57, 168–171.

Gaudin, E., Simon, M., Quijada, J., Schelcher, F., Lespine, A., Sutra, J.F., Hoste, H., 2016. Effects of sainfoin (*Onobrychis viciifolia*) pellets on a multi resistant strain of *Haemonchus contortus* and on the efficacy of oral ivermectin. Vet. Parasitol. 227, 122–129.

George, M.M., Lopez-Soberal, L., Storey, B.E., Howell, S.B., Kaplan, R.M., 2018. Motility in the L3 stage is a poor phenotype for detecting and measuring resistance to avermectin/milbemycin drugs in gastrointestinal nematodes of livestock. Int. J. Parasitol. Drugs Drug Resist. 8, 22–30.

Ghisi, M., Kaminsky, R., Mäser, P., 2007. Phenotyping and genotyping of *Haemonchus contortus* isolates reveals a new putative candidate mutation for benzimidazole resistance in nematodes. Vet. Parasitol. 144, 313–320.

Githigia, S.M., Thamsborg, S.M., Larsen, M., Kyvsgaard, N., Nansen, P., 1997. The preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. Int. J. Parasitol. 27, 931–939.

Gómez-Rincón, C., Uriarte, J., Valderrábano, J., 2006. Efficiency of *Duddingtonia flagrans* against Trichostrongyle infections of sheep on mountain pastures. Vet. Parasitol. 141, 84–90.

Gómez-Rincón, C., Uriarte, J., Valderrábano, J., 2007. Effect of nematophagous fungus *Duddingtonia flagrans* and energy supplementation on the epidemiology of naturally infected kids. Vet. Res. 38, 141–150.

Gonzalez, J.F., Hernandez, J.N., Machin, C., Perez-Hernandez, T., Wright, H.W., Corripio-Miyar, Y., Price, D.R.G., Matthews, J.B., McNeilly, T.N., Nisbet, A.J., 2019. Impacts of breed type and vaccination on *Teladorsagia circumcincta* infection in native sheep in gran Canaria. Vet. Res. 50, 29.

Grace, D., Himstedt, H., Sidibe, I., Randolph, T., Clausen, P.H., 2007. Comparing FAMACHA© eye color chart and hemoglobin color scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. Vet. Parasitol. 147, 26–39.

Greer, A., Kenyon, F., Bartley, D.J., Jackson, E.B., Gordon, Y., Donnan, A.A., McBean, D.W., Jackson, F., 2009. Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. Vet. Parasitol. 164, 12–20.

Greer, A.W., Van Wyk, J.A., Hamie, J.C., Byaruhanga, C., Kenyon, F., 2020. Refugia-based strategies for parasite control in livestock. Vet. Clin. N. Am. Food Anim. Pract. 36, 31–43.

Grønvold, J., Wolstrup, J., Nansen, P., Henriksen, S.A., Larsen, M., Bresciani, J., 1993. Biological control of nematode parasites in cattle with nematode trapping fungi: a survey of danish studies. Vet. Parasitol. 48, 311–325.

Haehling, M.B., Toscano, J.H.B., Giraldelo, L.A., Santos, I.B., Esteves, S.N., Benavides, M.V., Tholon, P., Chagas, A.C.S., 2021. Is selection for resistance and resilience to *Haemonchus contortus* possible in Morada Nova sheep? Sm. Rumin. Res. 201, 106447.

Haftu, M.G., Meresa, M., Kebede, E., Hadush, B., 2020. Review on anthelmintic resistances and its detection method on gastrointestinal nematodes of small ruminants. J. Anim. Sci. Vet. Med. 5, 30–36.

Hayward, A.D., Nussey, D.H., Wilson, A.J., Berenos, C., Pilkington, J.G., Watt, K.A., Pemberton, J.M., 2014. Natural selection on individual variation in tolerance of gastrointestinal nematode infection. PLoS Biol. 12, e1001917.

Healey, K., Lawlor, C., Knox, M.R., Chambers, M., Lamb, J., 2018a. Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: tracer studies in sheep. Vet. Parasitol. 253, 48–54.

Healey, K., Lawlor, C., Knox, M.R., Chambers, M., Lamb, J., Groves, P., 2018b. Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: pasture larval studies in horses, cattle and goats. Vet. Parasitol. 258, 124–132.

Hodgkinson, J., Kaplan, R.M., Kenyon, F., Morgan, E.R., Park, A.W., Paterson, S., Babayan, S.A., Beesley, N., Britton, C., Chaudry, U., Doyle, S.R., Ezenwa, V.O., Fenton, A., Howell, S.B., Laing, R., Mable, B.K., Matthews, L., McIntyre, J., Milne, C.E., Morrison, T.A., Prentice, J.C., Sargison, N.D., Williams, D.J.L., Wolstenholme, A.J., Devaney, E., 2019. Refugia and anthelmintic resistance: concepts and challenges. Int. J. Parasitol. Drugs Drug Resist. 10, 51–57.

Hogberg, N., Hessle, A., Lidfors, L., Enweji, N., Höglund, J., 2021. Nematode parasitism affects lying time and overall activity patterns in lambs following pasture exposure around weaning. Vet. Parasitol. 296, 109500.

Höglund, J., Dahlstrom, F., Sollenberg, S., Hessle, A., 2013. Weight gain-based targeted selective treatments (TST) of gastrointestinal nematodes in first-season grazing cattle. Vet. Parasitol. 196, 358–365.

Höglund, J., Enweji, N., Gustafsson, K., 2020. First case of monepantel resistant nematodes of sheep in Sweden. Vet. Parasitol. Reg Stud Reports 22, 100479.

Höglund, J., Mitchell, G., Kenyon, F., Skuce, P., Charlier, J., 2021. Anthelmintic resistance in ruminants: from research to recommendations. In: Meeting report of the 4th COMBAR Joint Working Group Meetings. 20 pp. Accessed on 10/08/2021 at https://www.combar-ca.eu/sites/default/files/MeetingSummary_4thWG.pdf.

Holm, A., Hill, R., Farsang, A., Jungbäck, C., 2019. Diagnostics in the veterinary field: the role in health surveillance and disease identification. Biologicals 61, 80–84.

Holzhauer, M., van Schaik, G., Saatkamp, H.W., Ploeger, H.W., 2011. Lungworm outbreaks in adult dairy cows: estimating economic losses and lessons to be learned. Vet. Rec. 169, 494.

Hoste, H., Martinez-Ortiz-de-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infection. Vet. Parasitol. 186, 18–27.

Hoste, H., Torres–Acosta, J.F.J., Sandoval-Castro, C.A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S.M., Terrill, T.H., 2015. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. Vet. Parasitol. 212, 5–17.

Inácio, S.V., Ferreira Gomes, J., Xavier Falcão, A., Nagase Suzuki, C.T., Bertequini Nagata, W., Nery Loiola, S.H., Martins Dos Santos, B., Soares, F.A., Rosa, S.L., Baptista, C.B., Borges Alves, G., Saraiva Bresciani, K.D., 2020. Automated diagnosis of canine gastrointestinal parasites using image analysis. Pathogens. 9, 139.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., Afaq, M., 2006. Anthelmintic resistance: the state of play revisited. Life Sci. 79, 2413–2431.

Jack, C., Hotchkiss, E., Sargison, N.D., Toma, L., Milne, C., Bartley, D.J., 2017. A quantitative analysis of attitudes and behaviours concerning sustainable parasite control practices from Scottish sheep farmers. Prev. Vet. Med. 139, 134–145.

Jackson, F., Coop, R.L., 2000. The development of anthelmintic resistance in sheep nematodes. Parasitology 120, S95–S107.

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Weber, S.S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Mäser, P., 2008. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452, 176–180.

Kaplan, R.M., 2020. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Vet. Clin. North Am. Food Anim. Pract. 36, 17–30.

Kaplan, R.M., Vidyashankar, A.N., 2012. An inconvenient truth: global worming and anthelmintic resistance. Vet. Parasitol. 186, 70–78.

Kenyon, F., Jackson, F., 2012. Targeted flock/herd and individual ruminant treatment approaches. Vet. Parasitol. 186, 10–17.

Kenyon, F., Dick, J.M., Smith, R.I., Coulter, D.G., McBean, D., Skuce, P.J., 2013. Reduction in greenhouse gas emissions associated with worm control in lambs. Agri 3, 271–284.

Kenyon, F., Hutchings, F., Morgan-Davies, C., van Dijk, J., Bartley, D.J., 2017. Worm control in livestock: bringing science to the field. Trends Parasitol. 33, 669–677.

Khan, S., Nisar, A., Yuan, J., Luo, X., Dou, X., Liu, F., Zhao, X., Li, J., Ahmad, H., Mehmood, S.A., Feng, X., 2020. A whole genome re-sequencing based GWA analysis reveals candidate genes associated with ivermectin resistance in *Haemonchus contortus*. Genes 11, 367.

Kipling, R.P., Bannink, A., Bartley, D.J., Blanco-Penedo, I., Faverdin, P., Graux, A.I., Hutchings, N.J., Kyriazakis, I., Macleod, M., Østergaard, S., Robinson, T.P., Vitali, A., Ahmadi, B.V., Özkan, Ş., 2021. Short communication: identifying key parameters for modelling the impacts of livestock health conditions on greenhouse gas emissions. Animal. 15, 100023.

Knox, M.R., Josh, P.F., Anderson, L.J., 2002. Deployment of *Duddingtonia flagrans* in an improved pasture system: dispersal, persistence, and effects on free-living soil nematodes and microarthropods. Biol. Cont. 24, 176–182.

Königová, A., Urda Dolinská, M., Babják, M., von Samson-Himmelstjerna, G., Komáromyová, M., Várady, M., 2021. Experimental evidence for the lack of sensitivity of in vivo faecal egg count reduction testing for the detection of early development of benzimidazole resistance. Parasitol. Res. 120, 153–159.

Kotze, A.C., Prichard, R.K., 2016. Anthelmintic resistance in *Haemonchus contortus*: history, mechanisms and diagnosis. Adv. Parasitol. 93, 397–428.

Kotze, A.C., Hunt, P.W., Skuce, P., von Samson-Himmelstjerna, G., Martin, R.J., Sager, H., Krücken, J., Hodgkinson, J., Lespine, A., Jex, A.R., Gilleard, J.S., Beech, R.N., Wolstenholme, A.J., Demeler, J., Robertson, A.P., Charvet, C.L., Neveu, C., Kaminsky, R., Rufener, L., Alberich, M., Menez, C., Prichard, R.K., 2014. Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. Int. J. Parasitol. Drugs Drug Resist. 4, 164–184.

Kotze, A.C., Gilleard, J.S., Doyle, S.R., Prichard, R.K., 2020. Challenges and opportunities for the adoption of molecular diagnostics for anthelmintic resistance. Int. J. Parasitol. Drugs Drug Resist. 14, 264–273.

Lacey, E., Gill, J.H., Craven, J.H., Redwin, J.M., 1995. Assays for the determination of anthelmintic resistance in nematode parasites WO9509246-A, 30.

Laurenson, Y.C., Kyriazakis, I., Forbes, A.B., Bishop, S.C., 2012. Exploration of the epidemiological consequences of resistance to gastro-intestinal parasitism and grazing management of sheep through a mathematical model. Vet. Parasitol. 189, 238–249.

Leathwick, D.M., Besier, R.B., 2014. The management of anthelmintic resistance in grazing ruminants in Australasia-strategies and experiences. Vet. Parasitol. 204, 44–54.

Levecke, B., Dobson, R.J., Speybroeck, N., Vercruysse, J., Charlier, J., 2012. Novel insights in the faecal egg count reduction test for monitoring drug efficacy against gastrointestinal nematodes of veterinary importance. Vet. Parasitol. 188, 391–396.

Levecke, B., Kaplan, R.M., Thamsborg, S.M., Torgerson, P.R., Vercruysse, J., Dobson, R.J., 2018. How to improve the standardization and the diagnostic performance of the fecal egg count reduction test? Vet. Parasitol. 253, 71–78.

Li, Y., Zheng, R., Wu, Y., Chu, K., Xu, Q., Sun, M., Smith, Z.J., 2019. A low-cost, automated parasite diagnostic system via a portable, robotic microscope and deep learning. J. Biophotonics 12, e201800410.

Little, P.R., Hodges, A., Watson, T.G., Seed, J.A., Maeder, S.J., 2010. Field efficacy and safety of an oral formulation of the novel combination anthelmintic, derquantel-abamectin, in sheep in New Zealand. N. Z. Vet. J. 58, 121–129.

Lu, Q., Liu, G., Xiao, C., Hu, C., Zhang, S., Xu, R.X., Chu, K., Xu, Q., Smith, Z.J., 2018. A modular, open-source, slide-scanning microscope for diagnostic applications in resource-constrained settings. PLoS One. 13, e0194063.

Macchi, C., Pomroy, W.E., Morris, R.S., Pfeiffer, D.U., West, D.M., 2001. Consequences of anthelmintic resistance on liveweight gain of lambs on commercial sheep farms. N. Z. Vet. J. 49, 48–53.

Maingi, N., Krecek, R.C., van Biljon, N., 2006. Control of gastro-intestinal nematodes in goats on pastures in South Africa using nematophagous fungi *Duddingtonia flagrans* and selective anthelmintic treatments. Vet. Parasitol. 138, 328–336.

Martínez-Valladares, M., Valderas-García, E., Gandasegui, J., Skuce, P., Morrison, A., Gómez, C., de Agüero, V., Cambra-Pellejà, M., Balaña-Fouce, R., Rojo-Vázquez, F.A., 2020. *Teladorsagia circumcincta* beta tubulin: the presence of the E198L polymorphism on its own is associated with benzimidazole resistance. Parasit. Vectors 13, 453.

McArthur, M.J., Reinemeyer, C.R., 2014. Herding the U.S. cattle industry toward a paradigm shift in parasite control. Vet. Parasitol. 204, 34–43.

McIntyre, J., Hamer, K., Morrison, A.A., Bartley, D.J., Sargison, N., Devaney, E., Laing, R., 2018. Hidden in plain sight–multiple resistant species within a strongyle community. Vet. Parasitol. 258, 79–87.

McKenna, P.B., 2002. Faecal egg counts as a guide for drench use. N. Z. Vet. J. 50, 123–124.

McKenna, P.B., 2013. Are multiple pre-treatment groups necessary or unwarranted in faecal egg count reduction tests in sheep? Vet. Parasitol. 196, 433–437.

McMahon, C., McCoy, M., Ellison, S.E., Barley, J.P., Edgar, H.W.J., Hanna, R.E.B., Malone, F.E., Brennan, G.P., Fairweather, I., 2013. Anthelmintic resistance in Northern Ireland (III): uptake of "SCOPS" (sustainable control of parasites in sheep) recommendations by sheep farmers. Vet. Parasitol. 193, 179–184.

Melville, L., Kenyon, F., Javed, S., McElarney, I., Demeler, J., Skuce, P., 2014. Development of a loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of *Haemonchus contortus* eggs in ovine faecal samples. Vet. Parasitol. 206, 308–312.

Melville, L.A., Redman, E., Morrison, A.A., Rebecca Chen, P.C., Avramenko, R., Mitchell, S., Van Dijk, J., Innocent, G., Sargison, F., Aitken, C., Gilleard, J.S., Bartley, D.J., 2020. Large scale screening for benzimidazole resistance mutations in *Nematodirus battus*, using both pyrosequence genotyping and deep amplicon sequencing, indicates the early emergence of resistance on UK sheep farms. Int. J. Parasitol. Drugs Drug Resist. 12, 68–76.

Melville, L.A., Hayward, A., Morgan, E.R., Shaw, D.J., McBean, D., Andrews, L., Morrison, A., Kenyon, F., 2021. Precision worm control in grazing lambs by targeting group treatment based on performance of sentinels. Animal 15, 100176.

Michel, J.F., 1969. The epidemiology and control of some nematode infections in grazing animals. Adv. Parasitol. 7, 211–282.

Miller, C.M., Waghorn, T.S., Leathwick, D.M., Candy, P.M., Oliver, A.B., Watson, T.G., 2012. The production cost of anthelmintic resistance in lambs. Vet. Parasitol. 186, 376–381.

Mohammedsalih, K.M., Krücken, J., Khalafalla, A., Bashar, A., Juma, F.R., Abakar, A., Abdalmalaik, A., Coles, G., von Samson-Himmelstjerna, G., 2020. New codon 198 β-tubulin polymorphisms in highly benzimidazole resistant *Haemonchus contortus* from goats in three different states in Sudan. Parasit. Vectors 13, 114.

Mohammedsalih, K.M., Krücken, J., Bashar, A., Juma, F.R., Abdalmalaik, A., Khalafalla, A., Abakar, A., Coles, G., von Samson-Himmelstjerna, G., 2021. Susceptible trichostrongyloid species mask presence of benzimidazole-resistant *Haemonchus contortus* in cattle. Parasit. Vectors 14, 101.

Moore, H., Pandolfi, F., Kyriazakis, I., 2016. Familiarity with and uptake of alternative methods to control sheep gastro-intestinal parasites on farms in England. Vet. Parasitol. 221, 1–8.

Morgan, E.R., Coles, G.C., 2010. Nematode control practices on sheep farms following an information campaign aiming to delay anthelmintic resistance. Vet. Rec. 166, 301–303.

Morgan, E.R., Hosking, B.C., Burston, S., Carder, K.M., Hyslop, A.C., Pritchard, L.J., Whitmarsh, A.K., Coles, G.C., 2012. A survey of helminth control practices on sheep farms in Great Britain and Ireland. Vet. J. 192, 390–397.

Morgan, E.R., Aziz, N.A., Blanchard, A., Charlier, J., Charvet, C., Claerebout, E., Geldhof, P., Greer, A.W., Hertzberg, H., Hodgkinson, J., Höglund, J., Hoste, H., Kaplan, R.M., Martínez-Valladares, M., Mitchell, S., Ploeger, H.W., Rinaldi, L., von Samson-Himmelstjerna, G., Sotiraki, S., Schnyder, M., Skuce, P., Bartley, D., Kenyon, F., Thamsborg, S.M., Vineer, H.R., de Waal, T., Williams, A.R., van Wyk, J.A., Vercruysse, J., 2019. 100 questions in livestock helminthology research. Trends Parasitol. 35, 52–71.

Morley, F.H.W., Donald, A.D., 1980. Farm management and systems of helminth control. Vet. Parasitol. 6, 105–134.

Morris, C.A., Bisset, S.A., Vlassoff, A., Wheeler, M., West, C.J., Devantier, B.P., Mackay, A.D., 2010. Selecting for resilience in Romney sheep under nematode parasite challenge, 1994-2007. New Zeal. J. Agric. Res. 53, 245–261.

Mravčáková, D., Váradyová, Z., Kopčáková, A., Čobanová, K., Grešáková, Ľ., Kišidayová, S., Babják, M., Dolinská, M.U., Dvorožňáková, E., Königová, A., Vadlejch, J., Cieslak, A., Ślusarczyk, S., Várady, M., 2019. Natural chemotherapeutic alternatives for controlling of haemonchosis in sheep. BMC Vet. Res. 15, 302.

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W.F., Salminen, J.P., Skøt, L., Smith, M.J., Thamsborg, S.T.M., Totterdell, P., Wilkinson, I., Williams, A.R., Azuhnwi, B.N., Baert, N., Grosse Brinkhaus, A., Copani, G., Desrues, O., Drake, C., Engström, M., Fryganas, C., Girard, M., Huyen, N.T., Kempf, K., Malisch, C., Mora-Ortiz, M., Quijada, J., Ramsay, A., Ropiak, H.M., Waghorn, G.C., 2018. Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. Crop. Sci. 58.

Nagamori, Y., Hall Sedlak, R., De Rosa, A., Pullins, A., Cree, T., Loenser, M., Larson, B.S., Smith, R.B., Goldstein, R., 2020. Evaluation of the VETSCAN IMAGYST: an in-clinic canine and feline fecal parasite detection system integrated with a deep learning algorithm. Parasit. Vectors 13, 346.

Nansen, P., Larsen, M., Grønvold, J., Wolstrup, J., Zorn, A., Henriksen, S.A., 1995. Prevention of clinical trichostrongylosis in calves by strategic feeding with predacious fungus *Duddingtonia flagrans*. Parasitol. Res. 81, 371–374.

Nielsen, M.K., 2021. What makes a good fecal egg count technique? Vet. Parasitol. 296, 109509.

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G., Waghorn, G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agr. Sci. 125, 281–289.

Niezen, J.H., Waghorn, G.C., Charleston, W.A.G., 1998. Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (*lotus pedunculatus*) or perennial ryegrass (*Lolium perenne*). Vet. Parasitol. 78, 13–21.

Njue, A.I., Prichard, R.K., 2003. Cloning two full-length beta-tubulin isotype cDNAs from *Cooperia oncophora*, and screening for benzimidazole resistance-associated mutations in two isolates. Parasitology 127, 579–588.

Odoi, A., Samuels, R., Carter, C.N., Smith, J., 2021. Antibiotic prescription practices and opinions regarding antimicrobial resistance among veterinarians in Kentucky, USA. PLoS One. 16, e0249653.

Olah, S., van Wyk, J.A., Wall, R., Morgan, E.R., 2015. FAMACHA©: a potential tool for targeted selective treatment of chronic fasciolosis in sheep. Vet. Parasitol. 212, 188–192.

O' Brien, D., Scudamore, J., Charlier, J., Delavergne, M., 2017. DISCONTOOLS: a database to identify research gaps on vaccines, pharmaceuticals and diagnostics for the control of infectious diseases of animals. BMC Vet. Res. 13, 1.

Palcy, C., Silvestre, A., Sauve, C., Cortet, J., Cabaret, J., 2010. Benzimidazole resistance in *Trichostrongylus axei* in sheep: long-term monitoring of affected sheep and genotypic evaluation of the parasite. Vet. J. 183, 68–74.

Panic, G., Duthaler, U., Speich, B., Keiser, J., 2014. Repurposing drugs for the treatment and control of helminth infections. Int. J. Parasitol. Drugs Drug Res. 4 (3), 185–200.

Paraud, C., Lumaret, J.P., Chartier, C., 2007a. Lack of effect of the nematophagous fungus *Duddingtonia flagrans* on the development of the dung beetle, *Aphodius constans*. Small. Rum. Res. 70, 276–279.

Paraud, C., Pors, I., Chartier, C., 2007b. Efficiency of feeding *Duddingtonia flagrans* chlamydospores to control nematode parasites of first-season grazing goats in France. Vet. Res. Commun. 31, 305–315.

Peña Espinoza, M., Valente, A., Thamsborg, S.M., Simonsen, H.T., Boas, U., Einemark, H.L., Lopez-Munoz, R., Williams, A.R., 2018. Anti-parasitic activity of chicory (*Cichorium intybus*) and the role of its natural bioactive compounds: a review. Parasit. Vectors 11, 475.

Peña Espinoza, M., Valente, A., Bornancin, L., Simonsen, H.T., Thamsborg, S.M., Williams, A.R., López-Muñoz, R., 2020. Anthelmintic and metabolomic analyses of chicory (*Cichorium intybus*) identify an industrial by-product with potent *in vitro* antinematodal activity. Vet. Parasitol. 280, 109088.

Peña-Espinoza, M., Boas, U., Williams, A.R., Thamsborg, S.M., Simonsen, H.T., Enemark, H.L., 2015. Sesquiterpene lactone containing extracts from two cultivars of forage chicory (*Cichorium intybus*) show distinctive chemical profiles and *in vitro* activity against *Ostertagia ostertagi*. Int. J. Parasitol. Drugs Drug Resist. 5, 191–200.
Peña-Espinoza, M., Thamsborg, S.M., Desrues, O., Hansen, T.V.A., Enemark, H.L., 2016. Anthelmintic effects of forage chicory (*Cichorium intybus*) against gastrointestinal nematode parasites in experimentally infected cattle. Parasitology 143, 1279–1293.
Peña-Espinoza, M., Williams, A.R., Thamsborg, S.M., Simonsen, H.T., Enemark, H.L., 2017. Anthelmintic effects of forage chicory (*Cichorium intybus*) against free-living and parasitic stages of *Cooperia oncophora*. Vet. Parasitol. 243, 204–207.
Phythian, C.J., Glover, M.J., 2019. Highs and lows of lambing time: sheep farmers' perceptions of the first outbreak of Schmallenberg disease in south West England on their well-being. Int. J. Environ. Res. Public Health 16, 5057.
Ploeger, H.W., Antonis, A.F.G., Verkaik, J.C., Vellema, P., Bokma-Bakker, M.H., 2016. Perceptions and actions of Dutch sheep farmers concerning worm infections. Vet. Parasitol. 229, 150–158.
Prichard, R.K., Hall, C.A., Kelly, I.C.A., Donald, A.D., 1980. The problem of anthelmintic resistance in nematodes. Austr. Vet. J. 56, 239–251.
Ramünke, S., Melville, L., Rinaldi, L., Hertzberg, H., de Waal, T., von Samson-Himmelstjerna, G., Cringoli, G., Mavrot, F., Skuce, P., Krücken, J., Demeler, J., 2016. Benzimidazole resistance survey for *Haemonchus*, *Teladorsagia* and *Trichostrongylus* in three European countries using pyrosequencing including the development of new assays for *Trichostrongylus*. Int. J. Parasitol. Drugs Drug Resist. 6, 230–240.
Ravinet, N., Bareille, N., Lehebel, A., Ponneau, A., Chartier, C., Chauvin, A., 2014. Change in milk production after treatment against gastrointestinal nematodes according to grazing history, parasitological and production-based indicators in adult dairy cows. Vet. Parasitol. 201, 95–109.
Ravinet, N., Lehebel, A., Bareille, N., Lopez, C., Chartier, C., Chauvin, A., Madouasse, A., 2017. Design and evaluation of multi-indicator profiles for targeted-selective treatment against gastrointestinal nematodes at housing in adult dairy cows. Vet. Parasitol. 237, 17–29.
Raza, A., Lamb, J., Chambers, M., Hunt, P.W., Kotze, A.C., 2016. Larval development assays reveal the presence of sub-populations showing high- and low-level resistance in a monepantel (Zolvix)-resistant isolate of *Haemonchus contortus*. Vet. Parasitol. 220, 77–82.
Richards, K.M., Cotton, S.J., Sandeman Richard, M., 2008. The use of detec- tor dogs in the diagnosis of nematode infections in sheep feces. J. Vet. Behav. Clin. Appl. Res. 3, 25–31.
Rinaldi, L., Veneziano, V., Morgoglione, M.E., Pennacchio, S., Santaniello, M., Schioppi, M., Musella, V., Fedele, V., Cringoli, G., 2009. Is gastrointestinal strongyle faecal egg count influenced by hour of sample collection and worm burden in goats? Vet. Parasitol. 163, 81–86.
Ritter, C., Adams, C.L., Kelton, D.F., Barkema, H.W., 2019. Factors associated with dairy farmers' satisfaction and preparedness to adopt recommendations after veterinary herd health visits. J. Dairy Sci. 102, 4280–4293.
Rodríguez, A.V., Goldberg, V., Viotti, H., Ciappesoni, G., 2015. Early detection of *Haemonchus contortus* infection in sheep using three different faecal occult blood tests. Open Vet. J. 5, 90–97.
Roeber, F., Jex, A.R., Gasser, R.B., 2013. Next-generation molecular-diagnostic tools for gastrointestinal nematodes of livestock, with an emphasis on small ruminants: a turning point? Adv. Parasitol. 83, 267–333.
Roos, M.H., Boersema, J.H., Borgsteede, F.H., Cornelissen, J., Taylor, M., Ruitenberg, E.J., 1990. Molecular analysis of selection for benzimidazole resistance in the sheep parasite Haemonchus contortus. Mol. Biochem. Parasitol. 43, 77–88.

Rose, H., Wang, T., Van Dijk, J., Morgan, E.R., 2015. GLOWORM-FL: a simulation model of the effects of climate and climate change on the free-living stages of gastro-intestinal nematode parasites of ruminants. Ecol. Model. 297, 232–245.

Rose Vineer, H., Morgan, E.R., Hertzberg, H., Bartley, D.J., Bosco, A., Charlier, J., Chartier, C., Claerebout, E., de Waal, T., Hendrickx, G., Hinney, B., Höglund, J., Ježek, J., Kašný, M., Keane, O.M., Martínez-Valladares, M., Letra Mateus, T., McIntyre, J., Mickiewicz, M., Munoz, A.M., Phythian, C.J., Ploeger, H.W., Vergles Rataj, A., Skuce, P.J., Simin, S., Sotiraki, S., Spinu, M., Stuen, S., Thamsborg, S.T.M., Vadlejch, J., Varady, M., von Samson-Himmelstjerna, G., Rinaldi, L., 2020. Increasing importance of anthelmintic resistance in European livestock: creation and meta-analysis of an open database. Parasite 27, 69.

Rosenstock, I.M., Strecher, V.J., Becker, M.H., 1988. Social learning theory and the health belief model. Health Educ. Behav. 15, 175–183.

Ruffell, A., Raza, A., Elliott, T.P., Kotze, A.C., 2018. The use of the larval development assay for predicting the in vivo efficacy of levamisole against *Haemonchus contortus* and *Trichostrongylus colubriformis*. Vet. Parasitol. 260, 6–11.

Ruiz-Huidobro, C., Sagot, L., Lugagne, S., Huang, Y., Milhes, M., Bordes, L., Prévot, F., Grisez, C., Gautier, D., Valadier, C., Sautier, M., Jacquiet, P., 2019. Cell grazing and *Haemonchus contortus* control in sheep: lessons from a two-year study in temperate Western Europe. Sci. Rep. 9, 12699.

Rumball, W., Skipp, R., Keogh, R., Claydon, R., 2003. 'Puna II' forage chicory (*Cichorium intybus* L.). New Zeal. J. Agr. Res. 46, 53–55.

Sales, N., Love, S., 2016. Resistance of *Haemonchus* sp. to monepantel and reduced efficacy of a derquantel / abamectin combination confirmed in sheep in NSW. Australia. Vet. Parasitol. 228, 193–196.

von Samson-Himmelstjerna, G., 2006. Molecular diagnosis of anthelmintic resistance. Vet. Parasitol. 136, 99–107.

von Samson-Himmelstjerna, G., Coles, G.C., Jackson, F., Bauer, C., Borgsteede, F., Cirak, V.Y., Demeler, J., Donnan, A., Dorny, P., Epe, C., Harder, A., Höglund, J., Kaminsky, R., Kerboeuf, D., Küttler, U., Papadopoulos, E., Posedi, J., Small, J., Várady, M., Vercruysse, J., Wirtherle, N., 2009. Standardization of the egg hatch test for the detection of benzimidazole resistance in parasitic nematodes. Parasitol. Res. 105, 825–834.

Sangster, N.C., 1999. Anthelmintic resistance: past, present and future. Int. J. Parasitol. 29, 115–124.

Santos, J., Vasconcelos, J.F., Frota, G.A., Freitas, E.P., Teixeira, M., Vieira, L., Bevilaqua, C., Monteiro, J.P., 2019. Quantitative molecular diagnosis of levamisole resistance in populations of *Haemonchus contortus*. Exp. Parasitol. 205, 107734.

Sargison, N., Scott, P., Jackson, F., 2001. Multiple anthelmintic resistance in sheep. Vet. Rec. 149, 778–779.

Sargison, N.D., Jackson, F., Bartley, D.J., Moir, A.C., 2005. Failure of moxidectin to control benzimidazole, levamisole- and ivermectin-resistant *Teladorsagia circumcinda* in a sheep flock. Vet. Rec. 156, 105–109.

Sargison, N.D., Wilson, D.J., Penny, C.D., Bartley, D.J., 2010. Unexpected production loss caused by helminth parasites in weaned beef calves. Vet. Rec. 167, 752–754.

Sargison, N.D., MacLeay, M., Morrison, A.A., Bartley, D.J., Evans, M., Chaudhry, U., 2019. Development of amplicon sequencing for the analysis of benzimidazole resistance allele frequencies in field populations of gastrointestinal nematodes. Int. J. Parasitol. Drugs Drug Resist. 10, 92–100.

Sargison, N.D., Mazeri, S., Gamble, L., Lohr, F., Chikungwa, P., Chulu, J., Hunsberger, K.T., Jourdan, N., Shah, A., Bailey, J.L.B., 2021. Conjunctival mucous membrane colour as an indicator for the targeted selective treatment of haemonchosis and of the general health status of peri-urban smallholder goats in southern Malawi. Prev. Vet. Med. 186, 105225.

Scales, G.H., Knight, T.L., Saville, D.J., 1995. Effect of herbage species and feeding level on internal parasites and production performance of grazing lambs. New Zeal. J. Agr. Res. 38, 237–247.
Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., Kaplan, R.M., Burke, J.M., Mosjidis, J.A., 2006. *Sericea lespedeza* hay as a natural deworming agent against gastrointestinal nematode infection in goats. Vet. Parasitol. 139, 150–157.
Silva, M.E., Braga, F.R., Borges, L.A., Oliveira, J.M., Lima, W.d.S., Guimarães, M.P., Araújo, J.V., 2014. Evaluation of the effectiveness of *Duddingtonia flagrans* and *Monacrosporium thaumasium* in the biological control of gastrointestinal nematodes in female bovines bred in the semiarid region. Vet. Res. Comm. 38, 101–106.
Silvestre, A., Cabaret, J., 2002. Mutation in position 167 of isotype 1 beta-tubulin gene of Trichostrongylid nematodes: role in benzimidazole resistance? Mol. Biochem. Parasitol. 120, 297–300.
Slusarewicz, P., Slusarewicz, J.H., Nielsen, M.K., 2021. Development and performance of an automated fecal egg count system for small ruminant strongylids. Vet. Parasitol. 295, 109442.
Small, A.J., Coles, G.C., 1993. Detection of anthelmintic resistance by culture *in vitro* of parasitic stages of ovine nematodes. Vet. Parasitol. 51, 163–166.
Springer, A., Jordan, D., Kirse, A., Schneider, B., Campe, A., Knubben-Schweizer, G., Müller, K.E., Hoedemaker, M., Strube, C., 2021. Seroprevalence of major pasture-borne parasitoses (Gastrointestinal nematodes, liver flukes and lungworms) in german dairy cattle herds, association with management factors and impact on production parameters. Animals (Basel) 11, 2078.
Stafford, K., Morgan, E.R., Coles, G.C., 2009. Weight-based targeted selective treatment of gastrointestinal nematodes in a commercial sheep flock. Vet. Parasitol. 164, 59–65.
Stromberg, B.E., Averbeck, G.A., 1999. The role of parasite epidemiology in the management of grazing cattle. Int. J. Parasitol. 29, 33–39.
Sukas, S., Van Dorst, B., Kryj, A., Lagatie, O., De Malsche, W., Stuyver, L.J., 2019. Development of a lab-on-a-disk platform with digital imaging for identification and counting of parasite eggs in human and animal stool. Micromachines. 10, 852.
Sutherland, I., Scott, I., 2010. Animal genetics and parasitism. In: Gastrointestinal Nematodes of Sheep and Cattle. Wiley Blackwell, ISBN: 978-1-4051-8582-0, pp. 195–204.
Sutherland, I.A., Bailey, J., Shaw, R.J., 2010. The production costs of anthelmintic resistance in sheep managed within a monthly preventive drench program. Vet. Parasitol. 171, 300–304.
Svensson, C., Lind, N., Reyher, K.K., Bard, A.M., Emanuelson, U., 2019. Trust, feasibility, and priorities influence Swedish dairy farmers' adherence and nonadherence to veterinary advice. J. Dairy Sci. 102, 10360–10368.
Sweeney, T., Hanrahan, J.P., Ryan, M.T., Good, B., 2016. Immunogenomics of gastrointestinal nematode infection in ruminants—breeding for resistance to produce food sustainably and safely. Parasit. Immunol. 38, 569–586.
Szewc, M., De Waal, T., Zintl, A., 2021. Biological methods for the control of gastrointestinal nematodes. Vet. J. 268, 105602.
Takeuchi-Storm, N., Thamsborg, S.M., Enemark, H.L., Boes, J., Williams, D., Denwood, M.J., 2021. Association between milk yield and milk anti-*Fasciola hepatica* antibody levels, and the utility of bulk tank milk samples for assessing within-herd prevalence on organic dairy farms. Vet. Parasitol. 291, 109374.
Taylor, M.A., Hunt, K.R., Goodyear, K.L., 2002. Anthelmintic resistance detection methods. Vet. Parasitol. 103, 183–194.
Terrill, T.H., Kaplan, R.M., Larsen, M., Samples, O.M., Miller, J.E., Gelaye, S., 2001. Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. Vet. Parasitol. 97, 261–268.

Tiwari, J., Kumar, S., Kolte, A.P., Swarnkar, C.P., Singh, D., Pathak, K.M., 2006. Detection of benzimidazole resistance in *Haemonchus contortus* using RFLP-PCR technique. Vet. Parasitol. 138, 301–307.

Torgerson, P.R., Paul, M., Furrer, R., 2014. Evaluating faecal egg count reduction using a specifically designed package "eggCounts" in R and a user friendly web interface. Int. J. Parasitol. 44, 299–303.

Torres-Acosta, J.F.J., Hoste, H., 2008. Alternative or improved methods to limit gastro-intestinal parasitism in grazing / browsing sheep and goats. Small. Rum. Res. 77, 159–173.

Tuersong, W., He, L., Zhu, T., Yang, X., Zhang, Z., Ahmad, A.A., Di, W., Wang, C., Zhou, C., Liu, H., Chen, J., Hu, M., 2020. Development and evaluation of a loop-mediated isothermal amplification (LAMP) assay for the detection of the E198A SNP in the isotype-1 β-tubulin gene of *Haemonchus contortus* populations in China. Vet. Parasitol. 278, 109040.

Umair, S., McMurtry, L.W., Knight, J.S., Simpson, H.V., 2016. Use of fluorescent lectin binding to distinguish eggs of gastrointestinal nematode parasites of sheep. Vet. Parasitol. 217, 76–80.

USDA, 2010. Beef 2007–08, Part IV: Reference of Beef Cow-Calf Management Practices in the United States, 2007–2008. National Animal Health Monitoring System, Fort Collins, CO.

Valente, A.H., de Roode, M., Ernst, M., Pena-Espinoza, M., Bornancin, L., Bonde, C.S., Martínez-Valladares, M., Ramünke, S., Krücken, J., Simonsen, H.T., Thamsborg, S.M., Williams, A.R., 2021. Identification of compounds responsible for the anthelmintic effects of chicory (*Cichorium intybus*) by molecular networking and bio-guided fractionation. Int. J. Parasitol. Drugs Drug Resist. 15, 105–114.

van der Voort M, Van Meensel J, Lauwers L, de Haan MH, Evers AG, Van Huylenbroeck G, Charlier J. Economic modelling of grazing management against gastrointestinal nematodes in dairy cattle. Vet Parasitol. 2017 Mar 15;236:68-75. doi: 10.1016/j.vetpar.2017.02.004. Epub 2017 Feb 11.

Van Wyk, J.A., 2001. Refugia—overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstepoort J. Vet. Res. 68, 55–67.

Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529.

Van Wyk, J.A., Malan, F.S., Randles, J.L., 1997. How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics? Vet. Parasitol. 70, 111–122.

Vande Velde, F., Claerebout, E., Cauberghe, V., Hudders, L., Van Loo, H., Vercruysse, J., Charlier, J., 2015. Diagnosis before treatment: identifying farmers' determinants for the adoption of sustainable practices in gastrointestinal nematode control. Vet. Parasitol. 212, 308–317.

Vande Velde, F., Hudders, L., Cauberghe, V., Claerebout, E., 2017. Changing farmers' behavior intention with a hint of wit: the moderating influence of humor on message sidedness. J. Environ. Psychol. 56, 97–103.

Vande Velde, F., Charlier, J., Hudders, L., Cauberghe, V., Claerebout, E., 2018a. Beliefs, intentions, and beyond: a qualitative study on the adoption of sustainable gastrointestinal nematode control practices in Flanders' dairy industry. Prev. Vet. Med. 153, 15–23.

Vande Velde, F., Charlier, J., Claerebout, E., 2018b. Farmer behaviour and gastrointestinal nematodes in ruminant livestock–uptake of sustainable control approaches. Front. Vet. Sci. 5, 255.

Vercruysse, J., Deprez, P., Everaert, D., Bassissi, F., Alvinerie, M., 2008. Breed differences in the pharmacokinetics of ivermectin administered subcutaneously to Holstein and Belgian blue calves. Vet. Parasitol. 152, 136–140.

Vercruysse, J., Charlier, J., Van Dijk, J., Morgan, E.R., Geary, T., Von Samson-Himmelstjerna, G., Claerebout, E., 2018. Control of helminth ruminant infections by 2030. Parasitology 145, 1655–1664.
Voinot, M., Cazapal-Monteiro, C., Hernández, J., Palomero, A., Arroyo Balán, F., Sanchís, J., Pedreira, J., Sánchez-Andrade, R., Paz-Silva, A., Arias, M., 2020. Integrating the control of helminths in dairy cattle: deworming, rotational grazing and nutritional pellets with parasiticide fungi. Vet. Parasitol. 278, 109038.
Walker, J.G., Ofthile, M., Tavolaro, M., Van Wyk, J.A., Morgan, E.R., 2015. Mixed methods evaluation of targeted selective anthelmintic treatment by resource-poor smallholder goat farmers in Botswana. Vet. Parasitol. 214, 80–88.
Waller, P.J., Echevarria, F., Eddi, C., Maciel, S., Nari, A., Hansen, J.W., 1996. The prevalence of anthelmintic resistance in nematode parasites of sheep in southern Latin America: general overview. Vet. Parasitol. 62, 181–187.
Walsh, T.K., Donnan, A.A., Jackson, F., Skuce, P., Wolstenholme, A.J., 2007. Detection and measurement of benzimidazole resistance alleles in *Haemonchus contortus* using real-time PCR with locked nucleic acid Taqman probes. Vet. Parasitol. 144, 304–312.
Wang, C., Torgerson, P.R., Kaplan, R.M., George, M.M., Furrer, R., 2018. Modelling anthelmintic resistance by extending egg counts package to allow individual efficacy. Int. J. Parasitol. Drugs Drug Resist. 8, 386–393.
West, D.M., Pomroy, W.E., Kenyon, P.R., Morris, S.T., Smith, S.L., Burnham, D.L., 2009. Estimating the cost of subclinical parasitism in grazing ewes. Small. Rum. Res. 86, 84–86.
Whittaker, J.H., Carlson, S.A., Jones, D.E., Brewer, M.T., 2017. Molecular mechanisms for anthelmintic resistance in strongyle nematode parasites of veterinary importance. J. Vet. Pharmacol. Ther. 40, 105–115.
Williams, A.R., Palmer, D.G., 2012. Interactions between gastrointestinal nematode parasites and diarrhoea in sheep: pathogenesis and control. Vet. J. 192, 279–285.
Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. PLoS One 9, e97053.
Williams, A.R., Peña-Espinoza, M., Boas, U., Simonsen, H.T., Enemark, H.L., Thamsborg, S.M., 2016. Anthelmintic activity of chicory (*Cichorium intybus*): *in vitro* effects on swine nematodes and relationship to sesquiterpene lactone composition. Parasitology 143, 770–777.
Wilson, L., Rhodes, A.P., Dodunski, G., 2015. Parasite management extension – challenging traditional practice through adoption of a systems approach. N. Z. Vet. J. 63, 292–300.
Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, N.C., 2004. Drug resistance in veterinary helminths. Trends Parasitol. 20, 469–476.
Woodgate, R.G., Love, S., 2012. WormKill to WormBoss- -can we sell sustainable sheep worm control? Vet. Parasitol. 186, 51–57.
Zajíčková, M., Nguyen, L.T., Skálová, L., Raisová Stuchlíková, L., Matoušková, P., 2020. Anthelmintics in the future: current trends in the discovery and development of new drugs against gastrointestinal nematodes. Drug Discov. Today 25, 430–437.

## Further reading

Ahren, D., Faedo, M., Rajastiekar, B., Tunlid, A., 2004. Low genetic diversity among isolates of the nematode-trapping fungus *Duddingtonia flagrans*: evidence for recent worldwide dispersion from a single common ancestor. Mycol. Res. 108, 1205–1214.
Avramenko, R.W., Redman, E.M., Windeyer, C., Gilleard, J.S., 2020. Assessing anthelmintic resistance risk in the post-genomic era: a proof-of-concept study assessing the potential for widespread benzimidazole-resistant gastrointestinal nematodes in north American cattle and bison. Parasitology 147, 897–906.

Cabaret, J., Benoit, M., Laignel, G., Nicourt, C., 2009. Current management of farms and internal parasites by conventional and organic meat sheep French farmers and acceptance of targeted selective treatments. Vet. Parasitol. 164, 21–29.
Coop, R.L., Sykes, A.R., Angus, K.W., 1982. The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. J. Agric. Sci. 98, 247–255.
Cringoli, G., Maurelli, M.P., Levecke, B., Bosco, A., Vercruysse, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Prot. 12, 1723–1732.
Kenyon, F., Rinaldi, L., McBean, D., Pepe, P., Bosco, A., Melville, L., Devin, L., Mitchell, G., Ianniello, D., Charlier, J., Vercruysse, J., Cringoli, G., Levecke, B., 2016. Pooling sheep faecal samples for the assessment of anthelmintic drug efficacy using McMaster and Mini-FLOTAC in gastrointestinal strongyle and *Nematodirus* infection. Vet. Parasitol. 225, 53–60.
Michel, J.F., 1976. The epidemiology and control of some nematode infections in grazing animals. Adv. Parasitol. 14, 355–397.
Ravinet, N., Chartier, C., Merlin, A., Chauvin, A., 2019. Influence de la conduite du pâturage sur le risque parasitaire lié aux strangles digestifs. Fourrages. 238, 153–160.
Rinaldi, L., Coles, G.C., Maurelli, M.P., Musella, V., Cringoli, G., 2011. Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. Vet. Parasitol. 177, 345–352.
Shaw, D.J., Vercruysse, J., Claerebout, E., Dorny, P., 1998. Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. Vet. Parasitol. 75, 115–131.