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Morgan, E., Aziz, Blanchard, Charlier, Charvet, Claerebout, Geldhof, Greer, Hertzberg, Hodgkinson, Hoglund, Hoste, Kaplan, Martinez-Valladares, Mitchell, Ploeger, Rinaldi, van Samson-Himmelstjerna, Sotiraki, ... Vercruysse, J. (2018). 100 Questions in Livestock Helminthology Research. *Trends in Parasitology*. Advance online publication. https://doi.org/10.1016/j.pt.2018.10.006

Published in:
Trends in Parasitology

Document Version:
Peer reviewed version

100 Questions in Livestock Helminthology Research

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Abstract

An elicitation exercise was conducted to collect and identify pressing questions concerning the study of helminths in livestock, to help guide research priorities. Questions were invited from the research community in an inclusive way. Of 385 questions submitted, 100 were chosen by online vote, with priority given to open questions in important areas that are specific enough to permit investigation within a focused project or programme of research. The final list of questions was divided into ten themes. We present the questions and set them briefly in the context of the current state of knowledge. Although subjective, results provide a snapshot of current concerns and perceived priorities in the field of livestock helminthology, and we hope will stimulate ongoing or new research efforts.

Key words:

Helminth parasite, nematode, trematode, livestock, anthelmintic resistance, research priorities

Introduction: towards inclusive identification of research priorities

The study of the helminth parasites of livestock is facing a period of rapid change. The availability of a series of highly effective and affordable anthelmintics from the 1960s onwards coincided with the intensification of animal production systems in many parts of the world. As a result, adequate control of helminths could be achieved on the majority of farms with existing scientific knowledge, reducing incentives for investment in further research [1]. Currently, however, the effectiveness of control is breaking down in various areas. Anthelmintic resistance (AR) is increasing worldwide in helminths of all livestock species, highlighting the reliance of modern food production on chemical control of pests and parasites, and threatening the sustainability of livestock production, especially in grazing systems [2-4]. At the same time, changes in weather and climate are making infection patterns less predictable, and fixed protocol-driven approaches to helminth control are consequently less reliable [5]. To counter these challenges, alternative methods for helminth control are being developed, including, for example, vaccines, biological control, bioactive forages, grazing management, selective breeding, and various ways of targeting treatment in response to indicators of parasite infection or its impacts [6]. Development and effective application of novel control approaches require a return to fundamental scientific research to underpin future advances in parasite management. This renaissance of interest in veterinary helminthology comes at a time when it might profitably harness an explosion of new technologies, arising from rapid advances in molecular biology and 'omics', predictive modelling and data mining, sensor technologies and other fields [1].

In order to address research challenges and opportunities in relation to animal diseases, including those caused by helminths in livestock, new formal groupings serve to augment existing collaborations and provide a platform for coordination, mainly at European level (Box 1). In some, experts are enlisted in structured gap analyses to stimulate research and

92 feed into priority-setting by funders and policy makers, as well as produce published outputs
93 [7,8]. In other cases, experts produce opinionated reviews on the state of the art and expound
94 a vision of the way forward [1,4,9]. These exercises are built on consensus, often among
95 those who have worked together over a sustained period to develop ideas and drive progress
96 in the field. While these approaches are undoubtedly useful, they tend to perpetuate dominant
97 current thinking, and potentially neglect marginal but promising suggestions.
98 Alternatives are possible. Inspired by previous attempts in ecology [10], we here consult
99 more widely across the research community to identify key current questions in livestock
100 helminthology, to motivate and guide new work. The number 100 was chosen such that
101 questions might be broad enough to be strategically important, yet focused enough to be
102 tackled within a single focused research project or programme [10]. We elicited questions
103 from as wide a base as possible within the discipline (Box 2), to reduce the influence of
104 expert views and established dogmas on the questions presented, and to allow for disruptive
105 and creative ideas. Further rounds of voting and organization followed, and here we list the
106 questions judged most meritorious by a broad panel of specialists. The ten sub-sections are
107 based on the questions received and were not decided beforehand, and text commentary
108 follows rather than precedes each series of questions, in keeping with the 'bottom-up' spirit
109 of the exercise. The sections are structured to progress in a general direction from processes
110 of infection, through impacts, to control through chemical and alternative means, and include
111 challenges across the spectrum of fundamental and applied research. While we make no
112 claim to this list being definitive or complete, it is a snapshot of what researchers in livestock
113 helminthology consider to be important and topical at this time, and we hope that it will
114 stimulate discussion, and renew energy in existing or novel directions.
115

116 **Section I: Helminth biology and epidemiology**

Hypobiosis

1. What determines emergence of arrested helminth stages in the host, e.g. termination of hypobiosis in gastrointestinal nematodes in ruminants or cyathostomins in horses, or end of the mucosal phase of ascarids in poultry?

Hypobiosis is important for perpetuation of helminth populations during adverse environmental conditions. While factors inducing hypobiosis are well described (e.g. cold or dry seasonal cues, or immunity), factors governing the period of inhibition and timing of emergence are poorly understood. Intrinsic parasite factors, host physiology, or seasonality may all play a role [11,12], but the biochemical basis for these is mostly unknown. New molecular methods, e.g. transcriptomics, may be useful to understand mechanisms of emergence from arrest [13]. Resulting knowledge may pave the way for new control options during a phase when the therapeutic arsenal is typically limited due to the very low metabolic activity of the hypobiotic stages.

Fecundity

2. What regulates egg production in female helminths and can it be suppressed sufficiently to provide an epidemiological advantage?
3. Will breeding for host resistance (low faecal egg counts) drive nematode adaptation towards increased fecundity to compensate?

Interference with female worm fecundity could contribute to helminth control, and would benefit from detailed mapping of influencing factors, like host dietary, physiological and immunological status, location in the host, and intrinsic parasite factors, e.g. genetic predisposition and environment-induced changes. For example, in *Haemonchus contortus*,

142 worm size is highly correlated with the number of eggs present in adult females, and egg
143 production is limited by host immune regulation [15]. Ability to target fecundity specifically,
144 and evolutionary responses of parasites to such a strategy, are therefore likely to be highly
145 dependent on other parasite traits as well as host factors.
146
147 *Parasite adaptation to new hosts*
148 4. To what extent is there an exchange of parasites between wild and domestic ruminants?
149 5. Does cross-grazing of cattle and small ruminants encourage gastrointestinal nematode
150 species to adapt and cross between hosts?
151
152 Gastrointestinal nematode (GIN) species tend to have a preferred host, but there is
153 considerable evidence to indicate transmission and adaptation between livestock species
154 (sheep/goat/cattle) and between livestock and wildlife when either co-grazed or grazed
155 alternately on the same pasture [15]. In farming systems, control by means of alternate
156 grazing with different host species has been reported to break down due to parasite adaptation
157 [16]. Older studies often lack genotyping and apparent infection across multiple host species
158 may therefore constitute different parasite subpopulations or even species with cryptic host
159 preferences, as with lungworms in deer [17]. Whether the impact of cross-transmission
160 between wildlife and livestock is likely to amplify or reduce pasture infectivity and thus
161 transmission to livestock is in general an open question and likely to be context-specific [18].
162 Untreated wildlife could, moreover, act as a source of *refugia* for drug-susceptible genotypes,
163 or alternatively transfer resistant parasites to new hosts or locations [19]. The net effect of
164 livestock-wildlife contact on helminth ecology and evolution is hard to predict.
165
166 *Effects of climate change on epidemiology*

6. How do parasitic worms respond to climatic change and what is their environmental plasticity?

7. What is the effect of climate and weather, especially drought, on the spatial distribution of infective helminth larvae on pasture and on the subsequent risk for grazing animals?

8. How is climate change affecting overwintering of nematodes in temperate areas?

9. Will climate change result in a change of helminth species in temperate environments or will the existing ones simply adapt?

10. Is the recent increase in the prevalence of rumen fluke in Europe a threat to livestock farming?

Climate changes may not only affect helminths directly (e.g. the external stages and induction of hypobiosis) but also via effects on availability of definitive or intermediate hosts or on habitats, and through land use in agriculture. In general, parasites tend to adapt to the changes happening around them by evolving. Adaptation may involve strain variation in phenology, within-genotype variation in key life history traits and host switching [20]. Parasites may spread their chances of infecting hosts across variable or changing environments. An example in livestock is the adaptive epidemiology of *Nematodirus battus*, previously having a single generation per year (spring infection), but more recently evolving a strategy of two generations per year, which is better suited to unpredictable spring weather [21]. Parallel work on microbes indicates that sensitivity to environmental variation is itself a trait that can evolve, conferring resilience to changing climates [22]. There is considerable scope to improve predictions and measurements of helminth responses to climate change, in terms of evolutionary as well as epidemiological dynamics, and to include helminths with indirect life cycles such as trematodes, in which adaptive changes in intermediate hosts might also be important. Differentiating climate change from other forces and proving its role in parasite

192 range expansion is not straightforward, either for apparently emerging parasites such as the
193 rumen fluke *Calicophoron daubneyi* [23] or for other helminths, and this undermines
194 attempts to predict future challenges to farming. Given the multiple interacting factors that
195 drive parasite epidemiology, research should embed parasitic disease in wider studies of
196 climate change mitigation and adaptation in livestock and mixed agricultural systems [24].
197
198 *Improved diagnostics for epidemiological monitoring*
199 11. Can we develop good ways to enumerate infective helminth stages on pasture?
200
201 Various methods have been extensively documented to recover infective stages of GINs and
202 flukes from herbage or tracer animals, followed by microscopic counting and identification
203 by morphological or molecular methods [25]. However, modern quantitative and qualitative
204 molecular methods have not been sufficiently adapted for rapid estimation of the level of
205 parasite challenge. Success would have clear applications to parasite management as well as
206 improving the feasibility of field studies to test epidemiological and evolutionary predictions.
207
208 **Section II: Economic and environmental impacts**
209 12. What is the true financial cost of helminth infection?
210 13. Is profitable livestock husbandry possible without chemical parasite control?
211 14. Does the control of helminths reduce net methane emission over the lifetime of a
212 ruminant?
213 15. How can environmental impacts of anthelmintics be properly measured, including on
214 non-target fauna, and ecosystem functioning and service provision?
215 16. What are the costs (financial, human and to animal welfare) of anthelmintic resistance?
216

Holistic economic estimates of helminth impacts

The established aim of helminth control is to reduce parasite burden to improve animal health and productivity. As a result, research has tended to focus on how novel parasite control approaches can achieve higher efficacy and optimise production. Today, increasing emphasis is being placed on the sustainability of livestock farming. Therefore, the use of all inputs needs to be accounted for in the production equation and the role of helminth infection needs to be clarified in terms of optimal farm resource allocation, as well as its environmental and economic impacts [26]. There is early evidence from experimental and field studies of the beneficial impacts of effective helminth control on reducing greenhouse gas emission intensity in grazing livestock [27-29]. The impact of helminth parasitism on water use efficiency also needs to be better understood. There is a need to extend these approaches to emerging and resurgent parasite species such as rumen fluke and to investigate the direct impacts of failure of control, for example as a result of anthelmintic resistance.

Costing environmental impacts of drugs and drug resistance

Side-effects of anthelmintics as a consequence of 'leakage' into the environment, such as on non-target fauna [30] and onward impacts on their ecology and ecosystem service provision [31] need to be better understood and balanced against the beneficial impacts of treatment. The direct costs of anthelmintic resistance include the cost of the ineffective drug, the labour wastage in administering the ineffective drug, and the failure of adequate control leading to reduced production of meat and milk on a per hectare and per animal basis. However, there likely are many other indirect economic and environmental impacts since more animals will be needed to produce the same amount of food [32]. Generating these insights and integrating them into economic frameworks has great potential to support sustainable helminth control programmes at farm, regional and national levels. Valuing

sustainability, and the economic benefits of helminth control in less monetised farming systems, remain challenging [33].

Section III: Effects on host behaviour and welfare

17. How can we measure the impact of helminth infections on livestock welfare?

18. How does parasitism affect animal behaviour?

19. Can we use changes in behaviour to identify those individuals that need treatment?

20. Can we select for host behaviour to control helminths?

21. Do ruminants self-medicate by selectively grazing plants with anthelmintic compounds?

22. Are animals better off and healthier with some worms, rather than none? Studies are biased towards negative effects on hosts, and neglect potentially positive outcomes at individual and population levels.

Measuring behavioural impacts of parasitism

Research into the impacts of helminth infections on the behaviour and welfare of livestock has largely focused on aspects of direct economic importance in ruminant livestock [34], and is lagging behind research into the behavioural and welfare impacts of parasites in other host-parasite systems [35]. The impact of subclinical helminth infection on host behaviour and welfare indicators remains largely understudied, perhaps in part because such subclinical effects can be hard to detect and difficult to separate from those of other disorders. Still, changes can be more objectively measured today using new technologies. Thus, advances in electronic technology (e.g. 3D accelerometers), offers novel tools to monitor and detect host welfare and behavioural responses to parasitism and to link these to targeted control efforts [36]. Further, positive behaviours that allow livestock to avoid or suppress infection, such as self-medication and selective grazing, may be identified as markers to selectively breed for

‘behavioural’ resistance [37]. The importance of behaviour as a defence strategy against GIN is recognized in goats [38], but empirical evidence for selectively breeding grazing animals to develop this trait is so far lacking.

Helminth infection is not necessarily negative

Studies to date focus on negative effects on hosts, and neglect potentially positive outcomes of helminth infections, such as regulatory roles at scales ranging from gut microbiomes and inflammation [39] to entire grazing systems [40]. Studies taking a more holistic view of the consequences of infection for individual and group health would be timely given changes in farming systems and increasing societal concern in many countries for the welfare and environmental costs of modern farming practices.

Section IV: Host–helminth-microbiome interactions

23. How do gastrointestinal parasites communicate in the gut?

24. How does interaction between different helminths in co-infection affect the immune system of the host and the development of disease?

25. Are there associations between animals' microbiomes and helminth communities, and do they matter?

26. Can the alteration of gut microbiota influence immunity to parasites in livestock, and vice versa?

27. To what extent do co-infections between helminths and other specific pathogens, e.g. liver fluke and bovine tuberculosis; gastrointestinal nematodes and paratuberculosis; lungworms and respiratory pathogens; influence health outcomes for livestock and human health?

Helminths interact with other infections but consequences vary

The ability of helminths to influence the host response and dictate disease outcomes of co-infections is an active area of research within parasitology [41], in which many questions remain unanswered. In classical co-infection scenarios, a co-evolutionary dynamic between the vertebrate host, helminths and microbiome is thought to result from complex adaptations of each of the three components [42]. Research into helminth-microbiota co-infections in livestock hosts is in its early stages, raising questions about whether a host's microbiome and helminth community interact and communicate, how any such interaction impacts on the host immune response to both natural infections and vaccines, and whether it can be manipulated to enhance host immunity. Inconsistencies exist between different studies, methodologies and approaches, but a growing body of evidence from humans and rodent model systems has identified helminth-associated changes in gut microbiota [43,44]. It remains to be established whether this occurs as a direct effect of the parasite itself or as a secondary effect driven by the host and its immune response, or perhaps both [44]. Clearly a better understanding of co-infections (in consideration also of different helminths, or of helminths and micro-organisms), the mechanisms they invoke, and, importantly, their impact on the health and productivity of livestock is required [45,46]. A systems biology approach, drawing insights from diverse host environments (e.g. including livestock and wildlife systems), pathogen combinations and stages of infection [41,44,47-49] offers promise to advance our knowledge and identify potential alternative strategies for parasite control. A truly holistic view would also include the impact that helminths and their control may have on other diseases and their detection, including zoonoses [50].

Section V: Host resistance, resilience and selective breeding

28. Have 60 years of intense anthelmintic use changed the relative susceptibility of livestock to parasites? In other words, are animals less robust than they used to be as a result of protection from the effects of parasites by drugs, thereby causing selection of higher-producing but more parasite-susceptible animals?

29. How can resilience and resistance of ruminants to helminths be measured and distinguished?

30. Is resistance, tolerance or resilience the best breeding objective to produce livestock that require less anthelmintic treatment? Under what circumstances should breeders aim for each?

31. Breeding for resilience (high production potential in spite of elevated faecal worm egg counts) could result in significantly increased pasture contamination over many years. What will the impact of higher challenges be on resilient individuals? Will the resilience break down above a certain threshold?

32. Can targeted selective treatment, e.g. using FAMACHA, be used to select for parasite resilience, especially among low-input traditional breeds?

33. In non-selective breeding systems, does targeted selective anthelmintic treatment support weak animals and lead to loss of resilience at herd or flock level?

34. What are the life-time trade-offs between immunity to helminths (resistance) and impacts on growth and production (resilience) in different livestock systems?

35. Which are the main differences between cattle, sheep and goats in terms of resistance or resilience to helminth infection?

36. Which genotypes of livestock hold natural resistance to helminths, and how can they be exploited in modern production systems?

37. Why are some animals more prone to heavy parasite burdens than others?

Selecting optimal host phenotypes is not straightforward

341 Variation in susceptibility to parasites is multifactorial. Differences clearly exist between host
342 species, and these differences seem to derive from the evolutionary forces in play with regard
343 to grazing behaviors and the climate and environment where different hosts evolved.
344 However, even within host species, genetics, faecal avoidance behaviour and immunological
345 differences exist [51,52]. Moreover, the timing of measurement is important in distinguishing
346 between resistant and resilient animals as, should immunity develop, animals may thereafter
347 display a mixture of both resistance and resilience. Resistance is undoubtedly favourable
348 when faced with a fecund or highly pathogenic parasite, such as *H. contortus* [53]. In
349 contrast, resilience is associated with larger body weights and greater growth in the face of
350 helminth challenge, and can be reliably assessed based on the number of treatments required
351 using a targeted selective treatment regime [54,55]. Resilience, when it involves greater
352 tolerance of infection, generally results in greater pasture contamination, but resilient animals
353 also by definition have a greater threshold of parasite challenge before incurring loss of
354 productivity [52]. Whether the long-term epidemiological benefits of resistance outweigh the
355 missed growth opportunities remains to be determined, although the risk of pasture
356 contamination becoming too great if resilience is selected will depend on the environment
357 and grazing management, both of which influence transmission within and between seasons.
358 There are undoubtedly physiological costs to resistance and the interplay of resistance vs.
359 resilience (or tolerance) may differ between different parasite species depending on their
360 pathogenicity. These distinctions are important because hosts that are best at controlling
361 parasite burdens are not necessarily the healthiest, but can have a positive impact on the herd
362 infection levels by decreasing pasture contamination. Ultimately, resistance and
363 resilience/tolerance will have different effects not only on the epidemiology of infectious
364 diseases, but also on host–parasite coevolution [56]. The pursuit of improved host responses
365 to parasitism through selective breeding therefore requires optimization across multiple

dimensions, including characteristics of the main parasites of concern now and in future, production aims and farm management system, and should guard against unintended consequences for co-infections.

Section VI: Development and detection of anthelmintic resistance

38. What is the relative importance of management *versus* environmental factors in determining the development of anthelmintic resistance in livestock?

39. How does animal movement affect the spread of helminth infections and anthelmintic resistance?

40. What changes in genes other than those encoding for the immediate drug target, such as transporters and drug metabolism, are involved in anthelmintic resistance?

41. What do we understand about the fitness costs of anthelmintic resistance and how can they be measured?

42. Has selection for drug resistance changed the pathogenicity of parasites?

43. Is there a link between the size of the *refugia* needed to slow or prevent anthelmintic resistance and the molecule and formulation used (e.g. persistent *versus* non-persistent)?

44. Can combination anthelmintic formulations be designed that are more effective and that limit resistance development?

45. Do differences in life history traits and reproductive strategy affect the risk for development of anthelmintic resistance?

46. What is the effect of long-lasting drug formulations such as moxidectin injections or benzimidazole boluses on the development of anthelmintic resistance in sheep, goats and cattle?

47. Is treatment of ectoparasites with macrocyclic lactone drugs an important driver of anthelmintic resistance in sheep and goats?

48. Are *in-vitro*/genetic/laboratory methods for detection of anthelmintic resistance desirable, reachable and applicable for all anthelmintic drug groups?

49. How can we best improve monitoring of the efficacy of current control methods (e.g. through diagnostics, resistance testing and surveillance)?

50. How useful are composite faecal egg counts to detect anthelmintic resistance?

51. What is the true status of anthelmintic resistance in less-studied livestock systems, e.g. ascarids in pigs and poultry?

52. Is there compelling genetic evidence for reversion to drug susceptibility under any circumstances?

53. How can the prevalence of anthelmintic resistance be practically measured in a way that minimises bias?

Mechanisms and processes in resistance

The evolution of AR in parasitic helminths is considered to be driven by a range of parasite intrinsic and extrinsic factors [57]. To the former belong drug- and species-specific susceptibility, effective parasite population size and genetic variability. External factors include treatment frequency and intensity, and the size of the *refugia*, which strongly depend on local management and environmental determinants. How these factors interact and influence the development of a phenotypically resistant worm population is currently largely unclear. Also the molecular mechanisms of AR are not well established for most combinations of helminth species and drug groups. Nevertheless, in the case of the benzimidazoles, a well-developed understanding of the resistance mechanism has enabled molecular tools to be established for AR detection, which can be used to elucidate patterns of spread of resistance on a broad scale for ruminants [58]. The situation in pigs and poultry, however, is barely known [59].

Towards better diagnosis of anthelmintic resistance

There is a great need to extend our knowledge on the driving forces of AR development, to establish field applicable and meaningful resistance detection tools, and hence to provide more up-to-date and reliable information on the occurrence of AR. In an era of revolution of technology in the diagnostic industries, improvement of the "old-fashioned" faecal egg count reduction test (FECRT), for example through use of pooled faecal samples [60-62], or eventually automation, has great potential to allow more rapid, labour-efficient and remote assessment of AR. This remains a worthwhile aim because definitive molecular tests remain elusive for most drug groups and helminth species. Better tests would enable AR to be distinguished from other causes of poor efficacy, including through the administration of sub-standard generic compounds [63]. Links between AR in livestock and humans, through zoonotic transmission of resistant parasites such as *Ascaris* spp., and in terms of potential for shared understanding of mechanisms and approaches to limit AR, remain underexplored.

Section VII: Practical management of anthelmintic resistance

When to intervene against resistance

54. What is the usefulness of anthelmintics working at decreased (e.g. 50% or 80%) efficacy?

55. When should drug combinations be used to combat anthelmintic resistance, and when not?

Optimal usage of anthelmintic drugs in the face of AR should be tailor-made and consider parasite species, host species, farm management and climatic factors [2,3]. Deciding how to extend the lifetime of drugs, either before or after some resistance is evident [64,65], requires consideration of actual levels of AR and how fast AR spreads given selection pressures

441 imposed by factors such as drug type and number of treatments, whether treatments are
442 targeted or not, and the presence of *refugia* [66,67].
443
444 *Refugia in principle and practice*
445 56. What empirical evidence is there that *refugia* slow down the development of drug
446 resistance?
447 57. What proportion of a helminth population must be left in *refugia* in order to slow the
448 development of anthelmintic resistance?
449 58. How does the level of *refugia* influence the detection and spread of resistant phenotypes
450 in different hosts, different parasites and different treatment systems?
451 59. Is there a role for *refugia* in control of liver fluke?
452 60. If *refugia* are not appropriate for all parasite species that display drug resistance, what
453 realistic alternatives exist for those situations?
454 61. Can anthelmintic resistance be practically reversed, e.g. through targeted selective
455 treatment, good grazing management, or reseeding (community replacement or dilution)
456 approaches?
457
458 The concept of *refugia* is widely accepted, but is still surrounded by several assumptions and
459 approximations, and the level of *refugia* required may depend on prevailing (e.g. climatic)
460 circumstances [68]. *Refugia* as a concept has been mainly applied to GIN but its role in
461 resistance management in other helminths needs further research. Also, the extent to which
462 *refugia* might play a role in the reversal of AR [65], as opposed to just slowing its
463 development [69] is currently far from clear, as is the practical usefulness of community
464 replacement strategies for re-gaining anthelmintic susceptibility on farms [70].
465

What to do about known resistance status?

62. What is the value of faecal egg count monitoring as a decision tool for anthelmintic treatments?

63. We are on the cusp of having molecular markers for drug resistance, e.g. for macrocyclic lactones in *Haemonchus contortus* and triclabendazole in liver fluke. How should we best apply them?

It has become common practice to apply blanket, whole-herd treatments without prior knowledge about infection levels or drug efficacy. To optimize drug usage, such prior knowledge appears to be requisite, and more science is required to create and evaluate new and more practical ways to measure levels of infection and AR.

Targeting treatments against helminths

64. Is targeted selective treatment sustainable in the long term, or will it decrease parasite overdispersion and hence ability to identify heavily infected individuals?

65. What are the most useful decision parameters in targeting anthelmintic treatments?

66. Is targeted selective treatment a feasible approach with which to control helminths with a very high biotic potential, e.g. the ascarids?

Animals within populations show different levels of susceptibility to infection both in terms of resilience and resistance, and parasites are typically over-dispersed within host groups. This opens up the path to employ targeted selective treatments of individual hosts, and in the process create and maintain *refugia* [6,69]. Treatment decision parameters need to be explored more fully; their applicability may depend on parasite species as well as host production system and much more empirical work is needed for optimisation.

491

492 *Reaching and influencing stakeholders to optimize helminth control*

493 67. Can we automate interpretation of data collected during targeted selective treatment, for
494 farmer decision support and also training?
495 68. How do we apply existing knowledge of the risk factors for anthelmintic resistance on
496 farms to effectively slow its development?
497 69. What are the characteristics of an optimal quarantine drench as a way of reducing the risk
498 of importing resistance with bought in animals?
499 70. How do we implement better dosing procedures of anthelmintics in cattle in order to
500 ensure therapeutic drug levels (pour-on vs. injection/oral)?
501 71. What practical steps should be taken on a farm when resistance to all known anthelmintic
502 drug classes develops?

503

504 Finally, although managing resistance through more effective targeting of treatment is an
505 intuitive approach that is becoming established best practice [6], challenges remain in terms
506 of fundamental understanding of the biological processes involved in AR. Furthermore, how
507 existing knowledge should best be integrated and structured for on-farm application, and
508 communicated effectively through farmer and expert advisory groups (e.g.
509 www.cattleparasites.org.uk; www.scops.org.uk; www.wormboss.com.au), itself needs a more
510 solid evidence base [9]. Effective uptake of alternative helminth management approaches
511 could not only delay AR, but also afford farmers more options if and when AR becomes
512 fixed, for example following efforts to dilute resistant alleles by introducing susceptible
513 worms [70].

514

515 **Section VIII: Vaccines and immunology**

72. Can the natural immune response to helminths be enhanced by applying a biological treatment (e.g. specific cytokine or cytokine inhibitor) and thereby control infections?

73. Do worms have a microbiome? Can it be exploited as a vaccine or treatment target?

74. How can vaccines against helminth infections in ruminants be integrated in control programmes?

75. In what ways do helminths resist or escape from the host immune system?

76. How well do anti-helminth vaccines have to work to be useful?

77. To what extent is the immunomodulation by helminth parasites detrimental to the animal's health when co-infections co-occur?

78. What mechanisms are involved in protective immunity against helminths?

79. What is the potential for a multivalent vaccine to control multiple species?

80. How are optimal helminth vaccination schedules influenced by infection pressure and can this be incorporated into decision making?

81. How fast do parasites adapt to increased immune selection pressures (for instance due to vaccines)?

More insight needed into natural immune responses

Helminths typically induce a T-helper 2 type immune response, but the effector mechanisms have not yet been elucidated and it is not always clear whether this immune response is host protective or to the advantage of the parasite, which is acknowledged as a major knowledge gap [8]. Incomplete knowledge about protective immune responses against helminths hampers vaccine development. Insight into the immune mechanisms would allow informed decisions about adjuvants and antigen delivery [71] and could lead to alternative immune therapies, e.g. cytokines or cytokine inhibitors, which has shown potential in porcine neurocysticercosis [72].

Integrating vaccines into control programmes

To be useful alternatives to anthelmintics, vaccines should protect against multiple helminth species [71]. At present, there is only one vaccine for gastrointestinal nematodes available; targeting *Haemonchus contortus* (http://barbervax.com.au/), and other experimental vaccines are also limited to single species and there is no evidence for cross-protection, e.g. between *Cooperia* and *Ostertagia* in cattle [73]. 'Multivalent' vaccines could also include those containing multiple antigens of a single parasite species, to avoid or slow down adaptation of the parasites to the vaccine, e.g. an experimental *Teladorsagia* vaccine in sheep that comprises multiple recombinant proteins [71]. To protect young animals until natural immunity has developed, vaccines should lower pasture infection levels by reducing worm egg output in vaccinated animals for a useful period [74]. The level and duration of protection needed will be different for different parasites and in different epidemiological settings, e.g. on pastures with high or low infection pressure, and may differ with changing climate or farm management.

Vaccination, even if only partially effective could become an important component of integrated worm control programmes, including pasture management and anthelmintic treatment [1]. The huge number of possible scenarios could be investigated using helminth transmission models [75-79]. After field validation, these models could ultimately lead to decision support software for integrated worm control [9]. The sustainability of vaccines, like anthelmintics, will depend on parasite evolution, and the ability of helminths to develop resistance to vaccine-induced host responses remains an open question.

Section IX: Alternative approaches to helminth management

Plant-based control

82. Many studies have shown a maximum efficacy of bioactive plant compounds around 60-70% reduction in gastrointestinal nematode burden: how can efficacy be driven higher? Is it needed?

83. Can different bioactive plants be combined to increase effects on gastrointestinal nematodes?

84. Can plants be cultivated for grazing that have maximum nutritive value and the potential to lower helminth burden?

85. How does processing and conservation of bioactive forages affect their efficacy?

86. What are the interactions between bioactive forages and synthetic anthelmintic drugs, *in vitro* and *in vivo*?

87. What are the mechanisms of action of bioactive plant compounds and metabolites in relation to parasite establishment and adult worm viability and fecundity?

88. What is the efficacy of plant based anthelmintics against drug resistant helminths?

With the increasing emergence of AR in helminths of livestock, alternative options are in demand, especially for the integrated control of GINs. Plants and their Secondary Metabolites (PSM) appear to be a promising option. Different PSM (e.g. tannins) have shown antiparasitic effects when used as nutraceuticals [80] or in phytotherapy [81]. Two hypotheses have been invoked to explain the anthelmintic properties of PSM [82]: pharmacological-like effects through disturbance of the parasite life-cycle [83], or indirect effects on the host immune response [84]. In both cases, more studies are needed to identify the mechanisms of action of PSM and their effect on helminth populations, including those with high levels of AR, as well as the potential role of PSM in managing helminths other than GINs. Feeding 'bioactive forages' can also improve nutrition and performance, and reduce GHG emissions, quite apart from any impacts on helminths.

591 The interactions between different PSM and between PSM and anthelmintics remain largely
592 unexplored and contrasting results have been described [85]. The development of refined
593 methods to assess the anthelmintic potential of plant compounds are needed. Some
594 practicalities around use of PSM on farms also need to be addressed, such as regulation of
595 mode of distribution, level of inclusion in feed, and potential residues in animal products.
596
597 *Other alternative control methods*
598 89. What are the main obstacles (not only technical) to the development of new technologies
599 to control helminths of livestock?
600 90. Can we target helminth stages outside the host to achieve control, e.g. killing stages
601 on pasture or manipulating intermediate host biology?
602 91. Are there basic processes in egg hatching or larval development that can be manipulated
603 to aid control?
604
605 The objective of integrated parasite management is to limit the level of parasitism below
606 acceptable limits while delaying the emergence of drug resistance. This aim has motivated
607 the search for and refined use of PSM as well as other alternatives to commercial chemical
608 anthelmintics, including vaccines, host resistance and grazing management [86]. Good
609 pasture management is one of the major means to limit the intake of infective larvae by
610 animals, e.g. by use of parasite-free fields, pasture rotations, and alternation of grazing
611 animals, taking into account the seasonal dynamics of helminth transmission. Manipulation
612 of environmental conditions that play a role in the development of intermediate stages may
613 also be a form of alternative control. For example, grazing away from wet pasture, where
614 feasible, markedly lowers the risk of *F. hepatica* infection, due to lower exposure to infection
615 near intermediate snail host habitats [87]. Free-living stages of GIN may also be targeted

616 directly, for instance through application of urea or other nitrogen-based fertilisers to pasture
617 [88,89]. Certain bioactive forages, e.g. chicory, are also thought to hamper the development
618 of free-living stages, either by reducing the fitness of eggs excreted from hosts grazing on the
619 forage, or because the physico-chemical properties of the forage reduce larval availability on
620 herbage [90]. Biological control based on nematode trapping fungi (*Duddingtonia flagrans*,
621 *Arthrobotrys musiformis*) or entomopathogenic bacteria can also reduce the number of free
622 living stages on pasture and the level of host infections; results from mechanical stressors
623 such as a diatomaceous earth are less promising [91,92]. Refined understanding of the
624 mechanisms of action of these non-chemotherapeutic alternative control methods and how
625 they might be applied to manage helminth populations on farms provide potentially fruitful
626 avenues for further research.
627
628 **Section X: Stakeholder engagement**
629 *New decision support tools for helminth control*
630 92. How can different novel control methods for helminths be integrated effectively and in a
631 way that is simple enough for farmers to implement?
632 93. Can helminth control decision support tools be integrated effectively in farm or pasture
633 management software?
634 94. How can we transfer automated technology to farmers, especially those that are resource-
635 poor?
636 95. Is research in veterinary helminth infections reaching livestock farmers in developing
637 countries and, if so, what is the impact?
638
639 Veterinary parasitologists working with livestock might consider extending their efforts from
640 task-oriented research targeting the development and refinement of helminth control

641 strategies, and advance towards advice-oriented health management practices. To achieve this
642 would involve answering some key research questions around development of decision
643 support tools that can integrate different worm control strategies into whole-farm
644 management [9], taking into account also the regulatory frameworks and economic
645 environments in which farmers operate. Researchers are now looking further down this road
646 and questioning how their strategies will fit best into the whole farm environment and how
647 decision tools can be integrated, for example in farm management practices and decision
648 support systems. Even though there is considerable knowledge on available complementary
649 strategies, substantial deficits remain around knowledge exchange and transfer, and the
650 research community is becoming increasingly aware that better promotion of such strategies
651 to the farmers is crucial for their success [93].
652
653 *Understanding farmer behaviour to support effective knowledge exchange*
654 96. What factors drive anthelmintic treatment decisions by farmers?
655 97. How can the importance of a strategic approach to helminth treatment be more effectively
656 promoted among producers, especially when drug resistance is not yet an issue?
657 98. What can we learn from social sciences to transfer knowledge on helminth control to
658 farmers?
659 99. How does the attitude of farmers with respect to accepting and implementing parasite
660 control measures differ between countries and cultures?
661 100. How will consumers influence livestock production practices, in terms of anthelmintic
662 use?
663
664 In order to develop control methods that are effectively applied, it is necessary to obtain
665 insights into factors that drive farmers' decisions about worm control and use those insights

666 to develop communication strategies to promote sustainable worm control practices [94].
667 Major reasons why suggested solutions often do not fit with farmers' views are that they are
668 highly complex (involving language and cultural barriers) and not cost-efficient (too
669 expensive), encompass conflicting interests (e.g. intensive versus extensive farming systems)
670 and priorities, and may require contradictory management interventions at farm level.
671 Consequently, educating and motivating farmers and adopting a multi-actor approach are key
672 issues. Stronger empirical evidence for the effectiveness of integrated parasite control
673 strategies and their compatibility with performance targets is key to adoption [94,95].
674 Researchers must understand the fundamental and instrumental relationships between
675 individual farmers' values, behaviour and perception of risk, to stimulate and qualify the
676 farmer's decision-making in a way that will increase the farmer's satisfaction and subjective
677 well-being, and not only narrow metrics around performance or financial return [26,96].
678 Factors that influence farmers' behaviour are not limited to technical or practical issues such
679 as ease of use or price, but also include less 'tangible' factors such as the opinion of others or
680 habits [97-99]. Barriers and incentives for sustainable worm control that were identified in
681 such quantitative and qualitative studies may vary between farmer types (e.g. sheep farmers
682 vs. dairy cattle farmers) or between countries. Moreover, before these factors can be
683 translated into communication strategies, they should first be validated in communication
684 experiments [100]. In the literature on changing animal health behaviour, the majority
685 comprises studies that investigate the factors that influence behaviour intention, which at best
686 suggests which social intervention could be developed to change this intended behaviour, but
687 rarely assess whether such intervention could work [101]. Finally, human behaviour (and thus
688 also farmer behaviour) is also strongly influenced by unconscious processes, such as
689 intuition, which has not yet been studied in the context of sustainable parasite control [102].

690 As a community, veterinary parasitologists need to adopt a trans-disciplinary approach,
691 together with epidemiologists, social scientists, economists and others (including livestock
692 scientists, grassland management experts, conservationists, processors, retailers and farmers
693 themselves), which will result in a better understanding of farmer behaviour and motivation
694 with respect to drug treatments and parasite control.
695
696 **Concluding remarks**
697 The questions listed above were the result of an attempt to elicit research priorities from a
698 wider constituency than in more usual review formats, which are typically led by a small
699 number of established experts. It was anticipated that this would yield a wider-ranging set of
700 potential research topics and directions, less constrained by forces that shape disciplinary
701 academic consensus. In the event, the topics and questions are broadly similar to those raised
702 in recent expert reviews [1,4,6-8,103], and reflect a high level of current concern over the
703 biology of AR, how to measure and manage it, and the quest for alternative options for the
704 control of helminths on farms. This is perhaps not surprising given that improved helminth
705 management is a key goal of most researchers in the discipline, whether they lean toward
706 fundamental or applied research, and that AR is the main threat to existing control strategies.
707 Control of helminth infections in mainstream farming systems with fewer chemical inputs is
708 a topical challenge and one that will require new research, technologies, and perhaps
709 economic goals [1].
710 Questions around helminth epidemiology, management of AR, and alternative control
711 approaches including *refugia*, were frequently repeated in the original list (see supplemental
712 material), for example being posed more than once for different parasite or host taxa. To
713 achieve feasible smaller research projects as envisaged at the start of this exercise, many of
714 the questions could be broken back down again to specific taxa, both to produce system-

715 specific knowledge and applied solutions, and to explore the generality of conclusions from
716 more studied contexts. Challenges in tropical or less developed countries yielded few specific
717 questions, as did those related to pig and poultry production. Participation was strongly
718 skewed towards European countries, in spite of efforts to be inclusive, possibly as a result of
719 the European roots of LiHRA, under whose auspices the exercise was conducted (Box 1).
720 Nevertheless, questions submitted from outside Europe focused on similar areas, and almost
721 all of the final questions are relevant across wide geographic areas and often globally. The
722 voting round (Box 2) might also have distorted results and led to the loss of original but less
723 popular ideas from the final list, though such a step was necessary to limit numbers of
724 questions and exclude some to which answers are already well-known. The full list is
725 included as supplemental material to this article.
726 While not definitive, the final list of 100 questions serves to indicate current concerns among
727 the livestock helminth research community, and highlights several areas in which existing
728 understanding is poor while fresh advances now appear possible. The questions might serve
729 to encourage or inspire work in those areas. For example, early career researchers might
730 peruse the list to identify topics on which short or starter projects might have
731 disproportionately high impact on the state of knowledge. It would be instructive to repeat
732 this exercise in future, to determine how many of the questions have been answered, and
733 whether the state of knowledge, the enabling technologies, or the problems of the day have
734 moved sufficiently to generate different gaps and priorities. In the meantime, as a community,
735 there is clearly work to be done to explore interesting questions whose answers are highly
736 relevant to the ability of humankind to feed itself in the future while respecting the global
737 environment and the health and welfare of the animals that sustain us.
738
739 **Acknowledgements**

We thank the officers and members of the Livestock Helminth Research Alliance (LiHRA) for encouraging this initiative and giving space to it in their annual meetings, to the World Association for the Advancement of Veterinary Parasitology for permitting elicitation of questions as part of their 26th biennial conference in Kuala Lumpur, Malaysia, and to the editors of Trends in Parasitology for commissioning this article. The authors credit the EU for funding leading to this work through FP7 STREP GLOWORM. We gratefully acknowledge Hassan Azaizeh, Sarah Beynon, Jacques Cabaret, Gerald Coles, Tina Alstrup Hansen, Alison Howell, Hamadi Karembe, Alvaro Martinez-Moreno, Francisco A. Rojo, Guillaume Sallé, Jože Starič, Eurion Thomas, and numerous anonymous participants for contributing generously to the exercise with questions and votes. This article is based in part upon work from COST Action COMBAR CA16230, supported by COST (European Cooperation in Science and Technology). MMV is funded by the Spanish "Ramón y Cajal" Programme, Ministry of Economy and Competitiveness (RYC-2015-18368), and ERM and HRV by UK BBSRC grant BB/M003949/1.

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999

BOX 1. Initiatives to identify and prioritise research needs on livestock diseases in Europe.

Deciding where public and private research spending will have the greatest impact is a complex process involving multiple interests. Often, *ad hoc* expert groups are created to provide decision makers with advice over specific topics. In addition, over the last decade several initiatives have emerged at European and global levels to foster international discussions and apply a structured approach to the identification of research gaps and priorities in the animal health domain, including livestock helminthology in Europe.

DISCONTOOLS (www.discontools.eu) is a publicly funded, open-access database to assist public and private funders of animal health research and researchers in identifying research gaps and planning future research [104]. The database contains research gaps as well as a gap scoring and prioritization model for more than 50 infectious diseases of animals. The information is provided by disease-specific expert groups and updated on a 5-year cycle.

The DISCONTOOLS database acts as a key resource for the STAR-IDAZ International Research Consortium on animal health (www.star-idaz.net), comprising research funders and programme owners from Europe, Asia, Australasia, the Americas, Africa and the Middle East, as well as international organisations, and includes representation from veterinary pharmaceutical companies. Members coordinate their research programmes to address agreed research needs, share results, and together seek new and improved animal health strategies for at least 30 priority diseases, infections or issues. These include candidate vaccines, diagnostics, therapeutics and other animal health products, procedures and key scientific information and tools to support risk analysis and disease control. STAR-IDAZ develops road maps on how to achieve these new animal health strategies.

The Animal Task Force (ATF) (www.animaltaskforce.eu) is a European public-private platform that fosters knowledge development and innovation for a sustainable and

competitive livestock sector in Europe. It represents key stakeholders from industry, farmers and research from across Europe. It is a knowledge-based lobby organisation working at the forefront of livestock related issues in Europe, including but not limited to animal health issues. The ATF unites members from every aspect of the livestock value chain (from feeding and breeding to production and processing), enabling an integrated approach to contribute to the environmental and societal challenges of livestock systems.

The Livestock Helminth Research Alliance (LiHRA) (www.lihra.eu) is a consortium of researchers that aims to develop sustainable effective helminth control strategies and promote their implementation by the livestock industry. LiHRA grew out of EU-funded research projects addressing challenges in the control of gastrointestinal nematodes (FP6 PARASOL) and liver fluke (FP6 DELIVER) in ruminants under global change (FP7 GLOWORM), and related projects investigating alternative control approaches (Marie-Curie Initial Training Networks NematodeSystemHealth, Healthy Hay and Legume Plus, www.legumeplus.eu). LiHRA meets annually to review current challenges, recent results and opportunities for collaborative research. Discussions within LiHRA gave rise to the current article, and also underpinned the EU-funded networking COST Action COMBAR.

BOX 2. An inclusive bottom-up elicitation of research priorities: approach and outcomes.

The questions presented in this article were elicited in a way intended to be inclusive and to encourage participation from a diverse range of researchers, regardless of career stage, gender or geographical location. Initially, LiHRA members (see Box 1) were introduced to the concept by oral presentation at their annual meeting in 2016 and asked to submit questions in hard copy or by email; this request was repeated by email to the wider alliance membership. A total of 151 questions were submitted in this way from 17 members, all based in Europe. To broaden geographic inclusivity, members were asked to forward the link to a simple online survey through their international networks, which introduced the exercise and requested questions by free text entry. An oral presentation was also made at the 26th biennial international conference of the World Association for the Advancement of Veterinary Parasitology (www.waavp.org), held in 2017 in Kuala Lumpur, Malaysia, and attended by >500 delegates from >50 countries, and again questions invited by completion of forms in hard copy on the day or by online survey. A further 28 questions from 9 people were submitted by hard copy, and 170 questions online from 32 people, following this exercise and an additional request at the LiHRA annual meeting in 2017. Finally, 36 questions were added from oral presentations at the WAAVP conference, having been identified by presenters as of pressing concern in their area of research. In total, 385 questions were submitted from at least 58 people (excluding secondary sources and conference presenters). Participants were based in at least 19 different countries, widely distributed across Europe and also including Malaysia, South Africa, Pakistan, the USA, Canada, and New Zealand. Elicitation through more specific organisations and interest groups was avoided in case of bias; for example, soliciting questions through the EU COST Action COMBAR, which focuses on combatting anthelmintic resistance in Europe, might have preferentially raised questions on this issue.

The master list was reduced to 100 questions by online vote. Those who submitted questions, and the wider LiHRA membership, were asked to award each question zero, one, two or three stars, with more stars awarded to questions considered of high general importance and well suited to guide a focused and feasible research project or programme. The objective was to identify questions in important areas that are novel and testable, rather than those that are open-ended, general or already known. This choice was made using personal judgement, and there was no limit to the total number of stars that could be awarded by each voter. Question order was randomized for each participant. In total, 38 people voted, from a similar geographic profile as that of question submitters, comprising 15 countries, of which 11 in Europe, with many claiming direct experience of work in a wider range of locations spanning five continents.

Questions were ranked according to total number of stars awarded, and in case of ties separated based on number of three-star scores awarded. When questions were repeated, effectively making the same point in a slightly different way, the highest scoring version was accepted, sometimes with minor changes to wording, others removed, and the next question on the list promoted into the top 100.

A core group was constituted from those who engaged most vigorously with the process, and to cover the breadth of subject areas raised, as well as to bring perspectives from across the world. The core group made minor edits to questions, and then reached a consensus through written discussion on the split into ten topic areas, which represented major themes in the submitted list. The final list was presented in these sub-sections, with ranks removed.

The methodology was adapted from earlier exercises in other subjects [10], modified to achieve greater global reach and less modification through repeated rounds of discussion. In this way, it was hoped that the final question list would capture a broad range of questions, unfiltered by expert opinion, relative to synthetic reviews. In the event, there was very little

1092 engagement from some parts of the world (e.g. Australia, South America) in spite of efforts
1093 to reach those regions, and a European bias in the core group and arguably therefore in the
1094 outcome, with a strong focus on anthelmintic resistance. The bias to Europe might be
1095 symptomatic of greater relevant research activity here than on other continents, but whatever
1096 the reason risks perpetuating focus on existing areas of strength in exactly the way this
1097 exercise sought to oppose. We exhort researchers in low and middle income countries in
1098 particular to seize the initiative in driving forward the research agenda to meet the needs in
1099 their countries, using researchers established elsewhere to support their efforts but not
1100 necessarily to determine the questions addressed or approaches used. It is also recommended
1101 that future elicitation exercises with similar aims make creative attempts to engage those who
1102 are less disposed to contribute, and further lessen the role of authors, for example by reducing
1103 the size and participation of the core group.
1104

Glossary

Anthelmintic – a chemical which can be used to control worm infections. Six different broad-spectrum classes are currently widely available for use in sheep (benzimidazoles, imidazothiazoles, tetrahydropyrimidines, macrocyclic lactones, amino acetonitrile derivatives, and spiroindoles) and four for cattle (benzimidazoles, imidazothiazoles, tetrahydropyrimidines and macrocyclic lactones). The terms drug, wormer, and de-wormer are commonly used synonyms.

Anthelmintic resistance – the heritable reduction in the sensitivity of helminths to anthelmintics when animals have been administered the correct dose of the drug, in the correct manner, using drugs that are within date and have been stored correctly.

Animal Task Force (ATF) (www.animaltaskforce.eu) - a European public-private platform that fosters knowledge development and innovation for a sustainable and competitive livestock sector in Europe. See Box 1.

Bioactive forages – crops or feedstuffs that reduce the numbers of worms in, or available to, a host. The effect can be either direct (anthelmintic activity; reduced survivability of free-living stages on pasture) or indirect (improved nutrition).

Biological control – the control of infection using other organisms or their natural products, such as nematophagous fungi (*Duddingtonia flagrans*) or crystal (CRY) and cytolytic (CYT) proteins of the soil borne bacterium *Bacillus thuringiensis*.

DISCONTOOLS – www.discontools.eu is a publicly funded, open-access database to assist public and private funders of animal health research and researchers in identifying research gaps and planning future research.

FAMACHA – FAffa MAllan CHArt –a colour-guide chart used to assess the degree of anaemia in an animal via the colour of their ocular membranes to determine the need for anthelmintic administration. Developed by three South African researchers (Drs Faffa Malan, Gareth Bath and Jan van Wyk) and named after one of the inventors.

Faecal Egg Count Reduction Test (FECRT) - a commonly used *in vivo* test to assess the efficacy of an anthelmintic through examination of egg counts of groups of animals pre- and post-anthelmintic administration. The reduction in faecal egg counts of treated animals is expressed as either a percentage reduction as compared to untreated control animals or using the treated animal as its own control (by comparing with the day-of-treatment count).

Host resilience – a host's ability to perform under parasite challenge.

Host resistance – a host's ability to control helminth infection, for example as illustrated by low worm burden or low faecal worm egg counts.

Hypobiosis – cessation in development of parasitic stages of roundworms within the host under unfavourable conditions, prior to resumption of development when conditions improve.

Integrated parasite management (IPM) – the use of a combination of multiple control methods (chemotherapeutic and alternatives) to sustainably control helminth infections.

Livestock Helminth Research Alliance (LiHRA) (www.lihra.eu) - a consortium of researchers that aims to develop sustainable effective helminth control strategies and promote their implementation by the livestock industry. See Box 1.

Plant secondary metabolites (PSM) – Plant products that are not directly involved in normal growth, development or reproduction, but instead are thought to be waste or stress products or defence mechanisms against herbivores and insects.

Refugia – parasite subpopulations from either the stages within the host or free-living stages that are not exposed to anthelmintic treatment, and that have the ability to complete their life cycle and pass on susceptible alleles to the next parasitic generation. This is generally achieved by ensuring that a proportion of the parasite population remains unexposed to drug, through either TT or TST (see below).

Star-IDAZ – International Research Consortium on animal health (www.star-idaz.net), comprising research funders and programme owners from Europe, Asia, Australasia, the Americas, Africa and the Middle East, as well as international organisations, and including representation from veterinary pharmaceutical companies. Members coordinate their research programmes to address agreed research needs, share results, and together seek new and improved animal health strategies for at least 30 priority diseases, infections or issues. See Box 1.

Targeted selective treatment (TST) – the treatment of only some individual animals within a group at one time, instead of the more common whole-group treatment, where all animals in the group are treated simultaneously.

Targeted treatment (TT) – treatment of animals at a time selected to either minimise the impact on the selection for anthelmintic resistance, or to maximise animal productivity.

Zoonoses – infections that can be transferred from animals to humans.

SUPPLEMENTAL MATERIAL

The full list of questions submitted, unedited, arranged in themes to reflect the manuscript.

Helminth biology and epidemiology

1. Are gastrointestinal nematodes transmitted from wild ruminants to domestic ones?
2. Are some species more or less pathogenic than they used to be?
3. Are there any new clinical techniques for the diagnosis of helminth infections of livestock?

4. Are there better ways of assessing parasite burden than WECs or weight gain?
5. Bovine lungworm – can we identify or better define risk factors/meteorological predictors of outbreaks of husk?
6. Can bio-marker detection system for helminths invasion detection be installed in milking robot, so the farm manager will immediately get access to this information?
7. Can co-occurrence of other host species (e.g. wildlife) reduce anthelmintic resistance in livestock by introducing non-AR helminths?
8. Can farm management be included dynamically in models of helminth dynamics under climate change?
9. Can increasing the diversity of species present in an individual reduce disease from any single species?
10. Can we develop good ways to enumerate larvae on pasture?
11. Can we genetically modify populations of helminths to a less prolific and pathogenic form that would modify wild populations of helminths to become less pathogenic?
12. Can we improve understanding of future risks (eg. climate change and drug resistance)?

13. Can wildlife remove infective stages from the environment and hence decrease parasite infection pressure for livestock?
14. Can you link parasite population dynamics to parasite population genetic structures, and subsequently to variability in parasite pathogenicity and life-history traits?
15. Do bio-markers in milk or saliva of livestock for early detection of helminth invasion that needs to be treated exist?
16. Do different species of GIN have different levels of impact?
17. Does a compatibility filter (as defined by Claude Combes) exist in terms of genome interaction between the parasite and the host?
18. Does AR affect helminth life histories outside of hosts?
19. Does cross-grazing of cattle and sheep encourage GI nematode species to adapt and cross between hosts?
20. Give three reasons why infections with helminths are still very important in livestock?

21. Have parasites with relatively long life-cycles been selected for shorter life cycles by frequent use of anthelmintics, as a parallel but independent selection process distinct from selection for drug resistance?
22. How are incoming *Ascaridia galli* larvae affected by either mucosal phase larvae and/or adult worms?
23. How are parasites evolving to deal with recent movement into climates very different from where they evolved over millions of years?
24. How can advances in parasite control be extended to less wealthy countries?
25. How can advancing high throughput technologies offers the prospect of progress in the area of applied parasitology?
26. How can free-living nematode stages survive on pastures?
27. How can helminths be managed on small farms with minimal grazing land?

28. How can we better practically detect and quantify viable liver fluke stages on pasture?

29. How can we better practically detect and species ID/profile GIN larvae on pasture?
30. How can we define the key features of new anthelmintics, taking into account user and environmental safety?
31. How can we effectively combine pasture management and parasite risk software?
32. How do free living stages of nematodes adapt to climate change?
33. How do infections with intestinal helminths affect the growth of young animals?
34. How do parasitic worms respond to climatic change and what is the environmental plasticity?
35. How do the different species of parasite present in an individual interact?
36. How do water management and grazing practices interact to determine infection rate with *Schistosoma* species in ruminants?
37. How does climatic change affect parasitism in grazing animals especially in semi-arid areas?
38. How harmful are tapeworms to sheep and goats?
39. How is climate change affecting overwintering of nematodes in temperate areas?
40. How is hypobiosis from ruminant GIN terminated?
41. How may massive anthelmintic chemotherapy in animal farming alter the life-traits of parasites?
42. How to control helminthiasis among small ruminants?
43. In co-grazing systems how often do cattle carry *Haemonchus contortus* and what are the consequences (biological and on weight gain or production)?
44. Is *Dicrocoelium dendriticum* a parasite worth combatting?
45. Is *Haemonchus* dominance really spreading in temperate areas and what difference should it make to worm control advice?
46. Is the epidemiology of lungworm (*Dictyocaulus viviparus*) changing – why so many outbreaks in older (dairy) animals?
47. Is the eradication of *Taenia solium* feasible?
48. Is the recent prevalence increase of rumen fluke in Europe a threat to livestock farming?

49. Should we really aim to eliminate GIN in grazing animals or had we better sustain them?

50. To what extent are we dealing with neglected parasites when we are examining faecal samples?
51. To what extent is extreme adaptation is considered genetic drift/shift in helminths?
52. To what extent is there an exchange of parasites between wild and domestic ruminants?

53. What are the dynamics of resumption of development of inhibited larvae in horses (cyathostomes)?
54. What are the emerging issues/diseases in helminthology?
55. What are the functional roles of genomic ‘non-coding’ dark matter?
56. What are the longitudinal infection dynamics of *Dictyocaulus viviparus* within a herd of supposedly immune cattle over a number of subsequent years?
57. What are the major factors affecting infection levels of grazing animals with helminths?

58. What are the major genomic changes that enable species to adapt to a warmer climate?

59. What are the paramount parameters to assess the morbidity due to helminth infections?

60. What are valid grounds on which to separate parasite species?
61. What do we understand about geographical differences and genetic variation in parasite populations?
62. What is the balance between drift and selection in gastro-intestinal nematode evolution?

63. What is the cause of the reduction in voluntary feed intake in parasitized animals?
64. What is the clinical relevance of AR in e.g. sheep or horses?
65. What is the demonstrable effect of climate change on helminth parasites of livestock (+ve or –ve)?
66. What is the difference in pathogenesis, effect on production, distribution and AR status between *Cooperia punctata*, *C. pectinata* and *C. oncophora*?
67. What is the effect of helminth infection on GHG emissions from livestock, either directly or indirectly?
68. What is the effect of weather/climate (especially drought) on the spatial distribution of GIN infective larvae on pasture and on the subsequent parasitical risk for grazing animals?

69. What is the efficient size of populations in gastrointestinal nematodes?
70. What is the empirical evidence that different parasites will respond on global climate change?
71. What is the epidemiology of *H. contortus* in northern Europe?
72. What is the genetic basis behind hypobiosis?
73. What is the impact of helminth parasitism in Europe in 2017?
74. What is the influence of global change in the dynamics of the epidemiology of GIN?
75. What is the inherent ability of a nematode to modulate its life-history traits to adapt to environmental pressures?
76. What is the pathogenic effect of rumen fluke?
77. What is the potential for parasite genomes? How should we use the information and what will they yield?
78. What is the prevalence of various helminthoses?
79. What is the relationship between parasitic diseases and the main infectious diseases of livestock?
80. What is the relevance of the wild animal - domestic animal interphase for the main parasitic diseases of livestock?
81. What is the role of wildlife in disseminating livestock parasites & AR
82. What is the spatial distribution of helminth infections and how are they interrelated?
83. What is the impact of anthelmintics on non-target fauna, functioning and ecosystem service provision?
84. What percentage of adult dairy and beef cattle carry worms or lesions from *Ostertagia* and what effect does this have on production?
85. When identifying wildlife reservoirs how much focus is put on identifying the direction of parasite transfer?
86. Where did *Calicophoron daubneyi* come from?
87. Which factors determine the length of the mucosal phase of *Ascaridia galli*?
88. Which helminth is more affected by climate change? Is it temperate or tropical? Why?

89. Which parasites will be the winners and losers according to climate change models?
90. Which user-friendly input data are required on a farm level to get useful output from a decision support tool or a transmission model?
91. Why do horses lack important groupings of parasites that are common in other grazing ungulates?

92. Will climate change result in a change of species in temperate environments or will the existing ones simply adapt?
93. What regulates egg production in females and can we suppress female egg production sufficiently to provide an epidemiological advantage?
94. Will breeding for resistance (low FECs and high production potential) drive nematode adaptation towards increased fecundity to compensate?

Helminth biology and epidemiology - diagnostics

95. How can I see or detect that my flock or herd is infected by helminths?
96. How can we improve the diagnosis of *Fasciola* spp?
97. How far are we away from tests in the live animal for immature fluke and Nematodirus infestations?
98. How to predict a clinical case of dictyocaulosis in cattle?
99. In a flock or herd, which sampling protocol should be followed for the diagnosis of helminth infections?
100. Is a mixed species of GINs in one animal difficult to control compared to an infected animal with one GIN species?
101. Is there some general European strategy for (manual) of examination of livestock for helminthoses, before a treatment? Which methods are used in particular countries?
102. What new technologies are used to detect infections by helminths in livestock?
103. When will automated diagnostic tools/technologies be really available for on-farm diagnosis?
104. Which user-friendly parameters can help the farmer (or veterinarian) to make informed decisions on helminth control in young stock?
105. Why are faecal egg counts not at all times a good parameter to assess worm counts of strongyles?

Economic and environmental impacts

106. From an economical and ecological point of view, what helminths do farmers think are the most important? How would they list them?
107. How accurately can we predict changes in the seasonality and magnitude of risk?
108. How can helminth control be integrated in farm management in a cost-efficient way?

109. How can we better assess production and health impacts of helminths?
110. How can you measure environmental impacts of anthelmintics?
111. Can we put an economic dollar value on the importance of a more strategic approach to GIN treatment to producers?
112. How does helminth control impact on the environment (MLs on microorganisms, environmental schemes etc)?
113. How important is it for us to chase subclinical GI nematodes in grazing beef cattle with low FEC?
114. How the three main farming systems (capitalistic, entrepreneur-type, peasant / small farming / family farming) modify through values and technicity the parasite community?
115. Is profitable livestock husbandry possible without chemical parasite control?
116. Is there a market space to promote livestock products raised without (or with limited) use of anthelmintics?
117. Is there an association between countries or regions that have high levels of *Fasciola* and level of income in those countries / regions?
118. Is there an impact in the environment by the overuse of anthelmintics over the past decades?

119. Should we be advising anthelmintic treatment of dairy cows with antibodies to *O. ostertagi* but no clinical signs? Is a potential 1kg/d increase in yield worth the cost, time and increased use of anthelmintics?
120. What are the consequences on productions of helminth infections (including pigs and poultry)?
121. What are the costs (financial, human and welfare) of anthelmintic resistance?
122. What are the economics of GIN and Fasciola infection in cattle?
123. What are the long-term impacts of anthelmintics on beneficial dung fauna and their functioning?
124. What is the economic burden of helminths of livestock in each country around the world, in 2017?
125. What is the economic impact of anthelmintic resistance in livestock?
126. What is the economical impact of strongyle infections in ruminants?
127. What is the real impact of parasitic gastroenteritis on small ruminant production?
128. What is the true financial cost of helminth infection?
129. What is the true on farm economic impact of sheep (and cattle?) bred for resistance and is it a viable option for future breeding? E.g. impact on reducing pasture contamination / subsequent parasite challenge?
130. Which factors determine the role of helminth infections in the whole-farm economic context?
131. Will the benefits of helminth control of livestock for global environmental sustainability become as important as economic benefits are now when promoting our research?
132. Does the control of helminths reduce the net methane emission over the lifetime of a ruminant?

Effects on host behaviour and welfare

133. Are animals better off and healthier with some worms, rather than none?
134. Can we select for host behaviour to control helminth infections?
135. Do ruminant parasites change the behaviour of the host?
136. Do ruminants graze complex vegetation selectively to avoid nematode infection?
137. Do ruminants self-medicate by selectively grazing plants with anthelmintic compounds?

138. How can parasites be beneficial to hosts (individually or in terms of population or species levels)? All studies are biased on the negative effect on host.
139. How can we develop animal production supportive and welfare based control strategies in soil-transmitted helminth infections?
140. How does parasitism affect animal behaviour and can we use changes in behaviour as a way of identifying those that need treatment?
141. How can we measure the impact of helminth infections on livestock welfare?

Host-helminth-microbiome interactions

142. Are there associations between animals' microbiomes and helminth communities?
143. Can the alteration of gut microbiota influence the immunity to parasites in livestock?
144. How does the gut microbiome interact with GI helminths and does it matter?
145. How important are other microorganisms and multispecies interactions for driving parasitic disease in livestock?
146. How is the pathobiome considered in the host genetic selection scheme?
147. How strong is the influence of microbiota on nematode diversity?
148. What is the importance of climate change, helminth infections and immune response to inter-current microbial infectious diseases?

149. How do co-infections with helminths, and other infective organisms influence impact on each other by direct or indirect immunologically related effects?
150. What is the role of co-infections e.g. bTB & fluke; ParaTB & GIN etc.?
151. What is the role of GIN in modifying the gut and lung microbiomes, and how does this impact risk of bovine respiratory disease?
152. How do host-parasite relationships evolve when the initial conditions are nearly (but not fully) the same: an application of the deterministic chaos of Poincaré?
153. How do GIN communicate in the GI tract?
154. How does interaction between different helminth species in co-infection affect the immune system of the host?

Host resistance / resilience and selective breeding

155. Are there any advantages to being an individual that is prone to high parasite burdens?

156. Breeding for resilience (high FECs and high production potential) could result in significantly increased pasture contamination over many years. What will the impact of higher challenges be on resilient individuals? Will the resilience break down above a certain threshold?
157. Can use of resilient sheep in a 'normal' flock (no Haemonchus) act as a source of susceptible nematodes?
158. Has 60 years of intense anthelmintic use changed the relative susceptibility of livestock to parasites? In other words, are animals wimpier than they used to be as a result of protection from the effects of parasites by drugs, thereby causing selection of higher producing but more parasite-susceptible animals?
159. How can genetic/gene manipulation be used in the parasite or the host to help with the control of helminths?
160. To what extent is the impact of strongylid infections in ruminants dependent on host resilience?
161. Under what circumstances should breeders aim for resilience, versus resistance, in livestock?
162. What impact will breeding of sheep for resistance and resilience to nematodes have on nematode challenge and adaptation?
163. Which are the main differences between cattle, sheep and goats in term of resistance/susceptibility to helminth infection?
164. Which genotypes of livestock hold natural resistance to helminths?
165. What do we understand about the fitness cost of resistance and how can it be measured?
166. Why are some animals more prone to heavy parasite burdens than others?
167. How to measure and distinguish the resilience and the resistance of ruminants infected with GIN?
168. Is resistance or tolerance a better breeding objective to produce small ruminants that require less anthelmintic treatment?
169. Can targeted selective treatment, e.g. using FAMACHA, be used to select for parasite resilience, especially among low-input traditional breeds?
170. In non-selective breeding systems, does TST support weak animals and lead to loss of resilience at herd or flock level?
171. What are the life-time trade-offs between immunity to helminths and impacts on growth and production, in different livestock systems?

Development and detection of anthelmintic resistance

172. Are data on drug failure/drug resistance within countries publicly available and are they reliable enough to be used as a mechanism to survey drug failure/resistance at a national / international level?
173. Are data related to helminth resistance available for particular European countries?
174. Can the use of combination drugs help to slow down the development of anthelmintic resistance?
175. Can we develop markers for susceptibility to ML anthelmintics?
176. Can we improve methods for monitoring efficacy of current control methods (e.g. surveillance, diagnostics and resistance testing)?
177. Can we replace worm egg counts with an on-farm 'colour-change', e.g. ELISA, technology?
178. Do combinatorial effects of different resistance mechanisms (i.e. target-associated and non-target-associated) exist and if so to what effect is this relevant in the field?
179. Do differences in life history traits and reproductive strategy affect the risk for development of anthelmintic resistance?
180. Do intra-ruminal bolus systems have an impact on the development of anthelmintic resistance?
181. Does copy number variation have a role in anthelmintic resistance?
182. Does gene duplication play a role in anthelmintic resistance?
183. Does selection by ivermectin preselect for moxidectin resistance?
184. Has the selection for drug resistance changed the pathogenicity of parasites?
185. How can the knowledge on AR in livestock be used to promote a better understanding of the development and mechanisms of AR in human GIN?
186. How can we design anthelmintic combinations that are more effective and that should/would limit resistance development?
187. How can we develop molecular markers for ML drugs?
188. How can we improve diagnostics: infection intensities and drug resistance?
189. How do we prevent anthelmintic resistance, when change makes it a moving target?
190. How does animal movement affect the spread of helminth infections and anthelmintic resistance?
191. How fast is AR developing in cattle nematodes?
192. How is size of refugia needed affected by the genetics of ML resistance?
193. How predictive can be a gastro-intestinal nematode model in terms of resistance appearance and emergence?
194. How useful are composite faecal egg counts to detect anthelmintic resistance?
195. *In-vitro*/genetic/lab methods for detection of anthelmintic resistances: desirable, reachable and applicable for all anthelmintic drug groups?
196. Is there evidence of selection for ML-R when treating for sheep scab?
197. Is treatment of ectoparasites with macrocyclic lactone drugs an important driver of anthelmintic resistance in sheep?
198. Practically, what should the percentage of sheep/goats/cows/heifers left untreated in a group to control the emergence of anthelmintic resistance?
199. What are the best diagnostic techniques to detect anthelmintic resistance?
200. What are the contributory factors for the development of anthelmintic resistance?
201. What are the key factors involved in the development of AH resistance, and mitigation measures?
202. What are the molecular mechanisms involved in resistance to macrocyclic lactones?
203. What are the prospects for identifying molecular markers for resistance?
204. What are the risk factors for multiple anthelmintic resistance development in cattle?

205. What changes in genes other than the immediate drug target, such as transporters and drug metabolism are involved in drug resistance?
206. What do genotype-phenotype studies tell us about the quantitative contribution of a particular mutation to the resistance phenotype?
207. What do we learn from the virtual absence of anthelmintic resistance in cattle?
208. What drugs are the cause of higher prevalence of anthelmintic resistance in cattle, sheep and goats?
209. What factors are involved in the development of anthelmintic resistance?
210. What factors drive the emergence of anthelmintic resistance?
211. What is the best way for in vivo quantitative evaluation of GIN burden in cattle?
212. What is the effect of long lasting moxidection injections of the development of ML resistance in sheep and cattle?
213. What is the empirical evidence for a lack of reversion to susceptibility when drug selection pressure is removed?
214. What is the global scenario of prevalence and optimal methods for detection of anthelmintic resistance in ruminants?
215. What is the key to molecular assays capable of detecting resistant worms?
216. What is the link between genetic variation and the risk for selection of resistance?
217. What is the relative importance of management versus environmental factors in determining the development of anthelmintic resistance in livestock?
218. What is the role of combination i.e. dual-active anthelmintics in current helminth control?
219. What is the role of sequencing (WGS/NGS) in understanding the genetic basis of AR in GIN & fluke?
220. What is the status of drug resistance in *Ascaris suum* and other important pig parasites?

221. What is the true, non-biased, prevalence of anthelmintic resistance?
222. What makes a parasite resistant to anthelmintics?
223. What role does the individual animal play in the development of drug resistance in a parasite population?
224. What specific genetic differences either cause resistance or are sufficiently closely associated with resistance to be able to serve as molecular markers?
225. Where are we at present in anthelmintic resistance in farm animals?
226. Which are the most rapid and accurate methods to detect the anthelmintic resistance?

227. Which are the newest anthelmintics available in the market, and is there any report about flock or herds resistant to these ones?
228. Which genes are implicated in the development of anthelmintic resistance according to the family of anthelmintic?
229. Why did AR (at least thus far) not occur in most gastro-intestinal helminths of dogs and cats?
230. Why is it so difficult to identify markers for genetic resistance?
231. Is there (genetic) evidence for reversion to susceptibility under any circumstances?

Practical management of anthelmintic resistance
232. Anthelmintic treatment and control programmes: where, who, when and how?
233. Are combination anthelmintics useful to combat anthelmintic resistance?
234. Are current control programmes suitable for helminths in livestock considering all or most of the productivity systems?

235. Can 'farmer's eye be used effectively to slow the development of AR in sheep flocks (it works but what about its effect on performance)?
236. Can we expect new anthelmintic compounds on the market in the (near) future?
237. How much are the major pharmaceutical companies investing in new anthelmintics, specifically?
238. We are on the cusp of having molecular markers for drug resistance e.g macrocylic lactone resistance in *Haemonchus contortus* and triclabendazole resistance in liver fluke. How should we best apply these markers?
239. Should focus on new drug discovery ensure the target is just one class of parasite so that resistance development due to inadvertent use can be minimised? E.g. if an injectable treatment for external parasites such as scab can be developed which doesn't also control roundworms.
240. What are the limitations for developing anthelmintic combinations?
241. What are the prospects for a new flukicide to treat immature/acute infection, especially in sheep?
242. What are the prospects for any novel anthelmintics, given experiences with new AADs & dual-actives?
243. What is the value of faecal egg count monitoring as a decision tool in anthelmintic treatments?
244. Is TST a feasible approach with which to control helminths with a very high biotic potential, e.g. the ascarids?
245. What reporting systems are in place to record drug failure/drug resistance within countries?
246. Could an anthelmintic-resistant flock or herd get back to be susceptible and how?
247. Describe the methods of integrated helminth parasite control?
248. Can we automate TST data interpretation, also for farmer training?
249. How can flukicides be applied more effectively, is refugia an option?
250. How can we make control more effective and sustainable?
251. How do we apply existing knowledge of the risk factors for anthelmintic resistance on farms to effectively slow its development?
252. How can we reverse AH resistance?
253. How do we implement better dosing procedures of anthelmintics to cattle in order to insure therapeutic drug levels (pour-on vs. injection/oral)?
254. How do we solve the conundrum of use of anthelmintic drug combinations – or when to use drug combinations and when not to?
255. How does the level of refugia influence the emergence of resistant phenotypes?
256. How to control anthelmintic resistance?
257. Is anthelmintic resistance genuinely irreversible or can susceptibility be restored within helminth populations?
258. Is deworming sheep or goats truly necessary?
259. Under what circumstances are combination drugs the answer to manage anthelmintic resistance?
260. What (empirical) evidence is there that refugia slows down the development of drug resistance?
261. What are the best strategies to prevent further spread of anthelmintic resistance (in small ruminants)?
262. What are the characteristics of an optimal quarantine drench as a way of reducing the risk of importing resistance with bought in animals?
263. What is the efficacy of mitigation measures to reduce non-target impacts of anthelmintic on the environment?

264. What is the optimal use of fasciolicides where there is triclabendazole resistance?
265. What is the role of refugia in slowing selection for AR in sheep/cattle GIN?
266. What is the usefulness of anthelmintics working at decreased (50% or 80%) efficacy?

267. What proportion of a parasite population must be left in refugia?
268. What steps should be taken when resistance to all known anthelmintic drug classes develops?
269. Is refugia relevant for all parasite species; if not, what realistic alternatives exist for those parasites that display drug resistance but for which refugia based control is not deemed appropriate?
270. What will be the best methods to control Fasciola in areas where there is free grazing?

271. Why is development of anthelmintic resistance not reversible, even in the absence of the specific drug?
272. Is targeted selective treatment sustainable in the long term?
273. Why is the (parasitological) community accepting strategic anthelmintic treatments against GIN in cows (not learning from the small ruminant example?
274. With good parasite management can on farm anthelmintic resistance be reversed? Especially to 2LV and 3ML classes of drugs as has been found in NZ?
275. Is there a link between the size of the refugia needed to prevent AR and the molecule used (persistent versus non persistent)?
276. How does the level of refugia influence the detection and spread of resistant phenotype in different hosts, different parasites and different treatment systems?
277. Is there a role for refugia in control of liver fluke?
278. What are the most useful decision parameters in targeted selective anthelmintic treatments?

Vaccines and immunology

279. Can we develop sustainable methods of control (eg. vaccines and management)?
280. Can we enhance the natural immune response to helminths by applying a biological treatment (e.g. specific cytokine or cytokine inhibitor) and thereby control them effectively?

281. Could immune-stimulatory drugs for livestock be used for combating helminths?
282. Does *Fasciola* modulate co-infection with other parasites?
283. Do worms have a microbiome? Can it be exploited as a vaccine or treatment target?
284. How are optimal helminth vaccination schedules influenced by infection pressure and can this be incorporated into decision making?
285. How can vaccines against helminth infections in ruminants be integrated in control programmes?
286. How can we develop and apply vaccines?
287. How does the parasite resist or escape from the host immune system?
288. How fast do parasites adapt to increased immune selection pressures (due to for instance vaccines)?
289. How may massive anthelmintic chemotherapy in animal farming alter host immunity structuration?
290. How well do anti-helminth vaccines have to work to be useful?
291. How would vaccines against soil-transmitted helminth infections influence population dynamics?
292. To what extent does overuse of/use of very effective anthelmintic products affect development of immunity to bovine lungworm?

293. To what extent is the immunomodulation by helminth parasites detrimental to the animal's health when co-infections co-occur?
294. What are the crucial effects that a vaccine against helminth(s) need to produce so that farmers agree to include them in their farm management?
295. What is the future for (recombinant) vaccines?
296. What is the future of vaccines against helminths of livestock?
297. What is the immunological difference between host species showing widely different responses to closely related parasite species (eg. cattle versus donkey with respect to *Dictyocaulus* spp.)?
298. What is the potential for a multivalent vaccine to control multiple species?
299. What is the potential for vaccines to control individual helminth species?
300. What mechanisms are involved in protective immunity against helminths?
301. What regulates egg production in females and can we suppress female egg production sufficiently to provide an epidemiological advantage?
302. Which efficacy is needed from a helminth vaccine and how can vaccination be integrated in sustainable parasite control?
303. Why don't we yet have vaccines to control helminth infections in livestock?
304. Why is the efficacy of the *Haemonchus* vaccine (hidden antigen approach) much lower in adult sheep?
305. Why is the protective immunity to *Ascaridia galli* limited or almost absent?

Alternative approaches to helminth management

306. Are there basic processes in egg hatching or larval development that can be manipulated to aid control?
307. Are there possible escaping mechanisms of GIN to alternative approaches (e.g. vaccines, bioactive compounds)?
308. As challenge increases, will this result in an increase in the proportion of the flock/herd needing treatment over time?
309. Can anthelmintic resistance be reversed through TST, good management or reseeding approaches?
310. Can different bioactive plants be combined to increase effects on GI nematodes?
311. Can knowledge of risk factors for nematode infection in cattle, derived from antibody testing, be used to target treatments more effectively within as well as between herds?
312. Can TSTs be applied to cattle or pig parasites?
313. Can we cultivate plants for grazing which have maximum nutritive value and the potential to lower helminth burden?
314. Can we manipulate the intermediate host (e.g. *Galba truncatula*) to help control *Fasciola hepatica* and *Calicophoron daubneyi*?
315. Can we use polyphenols or other natural compounds found in forage to control helminths of livestock?
316. Does a natural polyphenol causing 100% inhibition of L3 of GIN larvae *in vitro* represent a promising natural compound for integrated helminths control??
317. Does feeding of probiotics improve resistance to and outcome of GI helminth infection?

318. Does the inhibition of exsheathment of L3 stage of gastrointestinal nematodes represent a viable control method for these helminths?
319. How can investigation of tank milk be an attractive monitoring tool so that it can be used as a basis for intervention strategies?
320. How do we develop easy, on-farm tools (diagnosis) for the implementation of targeted selected treatments?

321. How does processing and conservation of bioactive forages affect their efficacy?
322. How is the pharmacokinetic behaviour of bioactive plant compounds in relation to parasitic nematodes situated in different body compartments (i.e. small intestine, large intestine, liver, lungs)?
323. How should vaccines be combined with anthelmintics to optimise control?
324. How successful are herbs as an alternative of anthelmintic to livestock helminth?
325. If reduced effectiveness of TST over time transpires, could targeted treatment instead of TST be used to minimise pasture contamination at strategic intervals e.g. every few years at a time of year when egg development success is greatest?
326. Is on-farm TST applicable in cattle viz-a-viz FAMACHA in sheep?
327. How can we practically target free-living gastrointestinal nematode stages outside the host?
328. Is TT (treating at times of highest risk) inherently incompatible with the aim of maximising refugia? E.g. by treating at the time when risk is highest (usually when development success is high) we are increasing the selection pressure.
329. Many studies have shown a maximum efficacy of bioactive (plant) compounds around 60-70% reduction – how do we get a higher efficacy? Is it needed?
330. Should TST be adapted to overall infection levels, such that whole-herd treatments are sometimes optimal?
331. To what extent should TST indicators for nematode infection be extended to include arthropod parasites?
332. What are the alternatives to anthelmintic drugs?
333. What are the interactions between bioactive forages and synthetic anthelmintic drugs, *in vitro* and *in vivo*?
334. How successful are herbs as an alternative of anthelmintic to livestock helminth?
335. What are the limitations of pasture management routines?
336. What are the mechanism of action of bioactive plant compounds and metabolites in relation to parasite establishment and adult worms?
337. What is effective worm control within a context of sustainability?
338. What is the best alternative to anthelmintics?
339. What is the effect of the use of alternative control measures (i.e. bioactive plants) as regards AH resistance?
340. What is the efficacy of alternative methods of livestock parasite control?
341. What is the efficacy of dung beetles for livestock helminth control?
342. What is the role of medicinal plants for developing new anthelmintics?
343. What should be the minimal size of a refugia population to ensure the efficacy of a TST strategy to prevent AR in ruminants?
344. Why does the *Duddingtonia* (BC) approach work less well in small ruminants?
345. Will TST result in increased pasture contamination over many years? Especially with increased overwinter survival of L3 on pasture.
346. What is the efficacy of plant based anthelmintics against drug resistant helminths?
347. What are the main obstacles to the development of new technologies to control helminths of livestock?

Stakeholder engagement

348. Are farmers able to adapt or do they need support (e.g. from predictive models)? Does this vary by sector e.g. dairy vs sheep?
349. Are farmers and/or vets from rural regions being well advised on what are the best practices for parasite control in their area?
350. Are our models any better than farmers' intuition?

351. Can veterinary surgeons get more involved in parasite control on sheep farms?

352. Can we convince producers to adopt more sustainable control practices (where resistance is not yet an issue; to prevent its development)?
353. How can different novel control methods for GI nematodes be integrated effectively and in a way that is simple enough for farmers to implement?
354. How can famer perceptions of anthelmintic resistance as something that happens to others be overcome to increase their efforts to combat it?
355. How can we better promote best practices of diagnosis and treatment for helminth control in livestock?
356. How can we improve uptake of sustainable parasite control measures by vets and farmers?
357. How can we increase correct management against parasitoses by livestock farmers?
358. How can we refine spatial granularity of farmers' data whilst protecting privacy?
359. How do we (the vet parasitology research community) achieve recognition for scientific papers that are aimed at practitioners, who do not publish themselves and therefore add nothing to citation rates?
360. Can we be more creative in delivering alternative control options to farmers, including in less developed countries?
361. How do we communicate the importance of a more strategic approach to GIN treatment to producers? Can we put an economic dollar value on it?
362. How does the attitude of farmers with respect to accepting and implementing parasite control measures differ between countries?
363. How sustainability are farmer out-reach projects on helminths?
364. How to improve the relationships (eg submission of shared projects) between Vet and Medical Helminthology (Parasitology)?
365. How will consumers influence livestock production practices, in terms of anthelmintic use?
366. How will farmers adapt to the impact of climate change (increased climate variability) on disease risk?
367. If tools were available to support farmers, what is the best way to encourage their use? Demonstration farms etc.?
368. In which direction can we improve evidence based medicine for helminth control by dairy veterinarians?
369. Is research in veterinary helminth infections reaching livestock farmers in developing countries and, if so, what is the impact?
370. Is the stronger regulation of the sale of anthelmintics the only current way to slow the continued development of anthelmintic resistance?
371. Vets, farmers, pharmaceuticals, researchers, stakeholders: which role for each one in the integrated control of parasites?
372. What are the treatment approaches currently applied by producers?
373. What factors drive anthelmintic treatment decisions by farmers?
374. What is the optimal way to deliver spatial decision support to farmers?
375. What is the role of human behaviour and psychology on livestock diseases?
376. What kind of practice from the farmer would help to get livestock free of helminths?
377. Why do most trust more on chemical parasite control than on adapting animal husbandry and grazing based on parasite life cycles?
378. Why does farmer uptake of crucially important recommendations fail?
379. Why we have been failing to achieve an integrated and sustainable helminth control programme?

380. Can we integrate helminth control decision support tools in farm management software?
381. How can we transfer automated technology to farmers, especially those that are resource-poor?
382. What can we learn from social sciences to transfer knowledge on helminth control to farmers?

Others

383. How can we best protect parasitology as a distinct discipline in 'systems-based' veterinary school curricula?
384. How do helminths infections in livestock impact stunting rates in children of subsistence farmers?
385. What is a helminth parasite?
386. What is the better way to fight these pests?
387. What is the effect of parasite control programmes on product quality and safety?
388. What is the European general treatment strategy of treatment of helminths in livestock? Which chemotherapeutics are used in particular countries?