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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.

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

117 *Hypobiosis*

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

121

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

131

132 *Fecundity*

133 2. What regulates egg production in female helminths and can it be suppressed sufficiently to
134 provide an epidemiological advantage?

135 3. Will breeding for host resistance (low faecal egg counts) drive nematode adaptation
136 towards increased fecundity to compensate?

137

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

worm size is highly correlated with the number of eggs present in adult females, and egg production is limited by host immune regulation [15]. Ability to target fecundity specifically, and evolutionary responses of parasites to such a strategy, are therefore likely to be highly dependent on other parasite traits as well as host factors.

Parasite adaptation to new hosts

4. To what extent is there an exchange of parasites between wild and domestic ruminants?
5. Does cross-grazing of cattle and small ruminants encourage gastrointestinal nematode species to adapt and cross between hosts?

Gastrointestinal nematode (GIN) species tend to have a preferred host, but there is considerable evidence to indicate transmission and adaptation between livestock species (sheep/goat/cattle) and between livestock and wildlife when either co-grazed or grazed alternately on the same pasture [15]. In farming systems, control by means of alternate grazing with different host species has been reported to break down due to parasite adaptation [16]. Older studies often lack genotyping and apparent infection across multiple host species may therefore constitute different parasite subpopulations or even species with cryptic host preferences, as with lungworms in deer [17]. Whether the impact of cross-transmission between wildlife and livestock is likely to amplify or reduce pasture infectivity and thus transmission to livestock is in general an open question and likely to be context-specific [18]. Untreated wildlife could, moreover, act as a source of *refugia* for drug-susceptible genotypes, or alternatively transfer resistant parasites to new hosts or locations [19]. The net effect of livestock-wildlife contact on helminth ecology and evolution is hard to predict.

Effects of climate change on epidemiology

- 167 6. How do parasitic worms respond to climatic change and what is their environmental
168 plasticity?
- 169 7. What is the effect of climate and weather, especially drought, on the spatial distribution of
170 infective helminth larvae on pasture and on the subsequent risk for grazing animals?
- 171 8. How is climate change affecting overwintering of nematodes in temperate areas?
- 172 9. Will climate change result in a change of helminth species in temperate environments or
173 will the existing ones simply adapt?
- 174 10. Is the recent increase in the prevalence of rumen fluke in Europe a threat to livestock
175 farming?

176

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

range expansion is not straightforward, either for apparently emerging parasites such as the rumen fluke *Calicophoron daubneyi* [23] or for other helminths, and this undermines attempts to predict future challenges to farming. Given the multiple interacting factors that drive parasite epidemiology, research should embed parasitic disease in wider studies of climate change mitigation and adaptation in livestock and mixed agricultural systems [24].

Improved diagnostics for epidemiological monitoring

11. Can we develop good ways to enumerate infective helminth stages on pasture?

Various methods have been extensively documented to recover infective stages of GINs and flukes from herbage or tracer animals, followed by microscopic counting and identification by morphological or molecular methods [25]. However, modern quantitative and qualitative molecular methods have not been sufficiently adapted for rapid estimation of the level of parasite challenge. Success would have clear applications to parasite management as well as improving the feasibility of field studies to test epidemiological and evolutionary predictions.

Section II: Economic and environmental impacts

12. What is the true financial cost of helminth infection?

13. Is profitable livestock husbandry possible without chemical parasite control?

14. Does the control of helminths reduce net methane emission over the lifetime of a ruminant?

15. How can environmental impacts of anthelmintics be properly measured, including on non-target fauna, and ecosystem functioning and service provision?

16. What are the costs (financial, human and to animal welfare) of anthelmintic resistance?

217 *Holistic economic estimates of helminth impacts*

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

230

231 *Costing environmental impacts of drugs and drug resistance*

232 Side-effects of anthelmintics as a consequence of ‘leakage’ into the environment, such as on
233 non-target fauna [30] and onward impacts on their ecology and ecosystem service provision
234 [31] need to be better understood and balanced against the beneficial impacts of
235 treatment. The direct costs of anthelmintic resistance include the cost of the ineffective drug,
236 the labour wastage in administering the ineffective drug, and the failure of adequate control
237 leading to reduced production of meat and milk on a per hectare and per animal basis.
238 However, there likely are many other indirect economic and environmental impacts since
239 more animals will be needed to produce the same amount of food [32]. Generating these
240 insights and integrating them into economic frameworks has great potential to support
241 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

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

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

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

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

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

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

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

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

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

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

339

340 *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].

416

417 *Towards better diagnosis of anthelmintic resistance*

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

430

431 **Section VII: Practical management of anthelmintic resistance**

432 *When to intervene against resistance*

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

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

436

437 Optimal usage of anthelmintic drugs in the face of AR should be tailor-made and consider
438 parasite species, host species, farm management and climatic factors [2,3]. Deciding how to
439 extend the lifetime of drugs, either before or after some resistance is evident [64,65], requires
440 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 reseedling (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

466 *What to do about known resistance status?*

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

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

472

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

477

478 *Targeting treatments against helminths*

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

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

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

484

485 Animals within populations show different levels of susceptibility to infection both in terms
486 of resilience and resistance, and parasites are typically over-dispersed within host groups.

487 This opens up the path to employ targeted selective treatments of individual hosts, and in the
488 process create and maintain *refugia* [6,69]. Treatment decision parameters need to be
489 explored more fully; their applicability may depend on parasite species as well as host
490 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**

- 516 72. Can the natural immune response to helminths be enhanced by applying a biological
517 treatment (e.g. specific cytokine or cytokine inhibitor) and thereby control infections?
518 73. Do worms have a microbiome? Can it be exploited as a vaccine or treatment target?
519 74. How can vaccines against helminth infections in ruminants be integrated in control
520 programmes?
521 75. In what ways do helminths resist or escape from the host immune system?
522 76. How well do anti-helminth vaccines have to work to be useful?
523 77. To what extent is the immunomodulation by helminth parasites detrimental to the
524 animal's health when co-infections co-occur?
525 78. What mechanisms are involved in protective immunity against helminths?
526 79. What is the potential for a multivalent vaccine to control multiple species?
527 80. How are optimal helminth vaccination schedules influenced by infection pressure and can
528 this be incorporated into decision making?
529 81. How fast do parasites adapt to increased immune selection pressures (for instance due to
530 vaccines)?

531

532 *More insight needed into natural immune responses*

533 Helminths typically induce a T-helper 2 type immune response, but the effector mechanisms
534 have not yet been elucidated and it is not always clear whether this immune response is host
535 protective or to the advantage of the parasite, which is acknowledged as a major knowledge
536 gap [8]. Incomplete knowledge about protective immune responses against helminths
537 hampers vaccine development. Insight into the immune mechanisms would allow informed
538 decisions about adjuvants and antigen delivery [71] and could lead to alternative immune
539 therapies, e.g. cytokines or cytokine inhibitors, which has shown potential in porcine
540 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

566 82. Many studies have shown a maximum efficacy of bioactive plant compounds around 60-
567 70% reduction in gastrointestinal nematode burden: how can efficacy be driven higher? Is it
568 needed?

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

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

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

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

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

578 88. What is the efficacy of plant based anthelmintics against drug resistant helminths?
579

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

The interactions between different PSM and between PSM and anthelmintics remain largely unexplored and contrasting results have been described [85]. The development of refined methods to assess the anthelmintic potential of plant compounds are needed. Some practicalities around use of PSM on farms also need to be addressed, such as regulation of mode of distribution, level of inclusion in feed, and potential residues in animal products.

Other alternative control methods

89. What are the main obstacles (not only technical) to the development of new technologies to control helminths of livestock?

90. Can we target helminth stages outside the host to achieve control, e.g. killing stages on pasture or manipulating intermediate host biology?

91. Are there basic processes in egg hatching or larval development that can be manipulated to aid control?

The objective of integrated parasite management is to limit the level of parasitism below acceptable limits while delaying the emergence of drug resistance. This aim has motivated the search for and refined use of PSM as well as other alternatives to commercial chemical anthelmintics, including vaccines, host resistance and grazing management [86]. Good pasture management is one of the major means to limit the intake of infective larvae by animals, e.g. by use of parasite-free fields, pasture rotations, and alternation of grazing animals, taking into account the seasonal dynamics of helminth transmission. Manipulation of environmental conditions that play a role in the development of intermediate stages may also be a form of alternative control. For example, grazing away from wet pasture, where feasible, markedly lowers the risk of *F. hepatica* infection, due to lower exposure to infection near intermediate snail host habitats [87]. Free-living stages of GIN may also be targeted

directly, for instance through application of urea or other nitrogen-based fertilisers to pasture [88,89]. Certain bioactive forages, e.g. chicory, are also thought to hamper the development of free-living stages, either by reducing the fitness of eggs excreted from hosts grazing on the forage, or because the physico-chemical properties of the forage reduce larval availability on herbage [90]. Biological control based on nematode trapping fungi (*Duddingtonia flagrans*, *Arthrobotrys musiformis*) or entomopathogenic bacteria can also reduce the number of free living stages on pasture and the level of host infections; results from mechanical stressors such as a diatomaceous earth are less promising [91,92]. Refined understanding of the mechanisms of action of these non-chemotherapeutic alternative control methods and how they might be applied to manage helminth populations on farms provide potentially fruitful avenues for further research.

Section X: Stakeholder engagement

New decision support tools for helminth control

92. How can different novel control methods for helminths be integrated effectively and in a way that is simple enough for farmers to implement?

93. Can helminth control decision support tools be integrated effectively in farm or pasture management software?

94. How can we transfer automated technology to farmers, especially those that are resource-poor?

95. Is research in veterinary helminth infections reaching livestock farmers in developing countries and, if so, what is the impact?

Veterinary parasitologists working with livestock might consider extending their efforts from task-oriented research targeting the development and refinement of helminth control

strategies, and advance towards advice-oriented health management practices. To achieve this would involve answering some key research questions around development of decision support tools that can integrate different worm control strategies into whole-farm management [9], taking into account also the regulatory frameworks and economic environments in which farmers operate. Researchers are now looking further down this road and questioning how their strategies will fit best into the whole farm environment and how decision tools can be integrated, for example in farm management practices and decision support systems. Even though there is considerable knowledge on available complementary strategies, substantial deficits remain around knowledge exchange and transfer, and the research community is becoming increasingly aware that better promotion of such strategies to the farmers is crucial for their success [93].

Understanding farmer behaviour to support effective knowledge exchange

96. What factors drive anthelmintic treatment decisions by farmers?

97. How can the importance of a strategic approach to helminth treatment be more effectively promoted among producers, especially when drug resistance is not yet an issue?

98. What can we learn from social sciences to transfer knowledge on helminth control to farmers?

99. How does the attitude of farmers with respect to accepting and implementing parasite control measures differ between countries and cultures?

100. How will consumers influence livestock production practices, in terms of anthelmintic use?

In order to develop control methods that are effectively applied, it is necessary to obtain 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].

As a community, veterinary parasitologists need to adopt a trans-disciplinary approach, together with epidemiologists, social scientists, economists and others (including livestock scientists, grassland management experts, conservationists, processors, retailers and farmers themselves), which will result in a better understanding of farmer behaviour and motivation with respect to drug treatments and parasite control.

Concluding remarks

The questions listed above were the result of an attempt to elicit research priorities from a wider constituency than in more usual review formats, which are typically led by a small number of established experts. It was anticipated that this would yield a wider-ranging set of potential research topics and directions, less constrained by forces that shape disciplinary academic consensus. In the event, the topics and questions are broadly similar to those raised in recent expert reviews [1,4,6-8,103], and reflect a high level of current concern over the biology of AR, how to measure and manage it, and the quest for alternative options for the control of helminths on farms. This is perhaps not surprising given that improved helminth management is a key goal of most researchers in the discipline, whether they lean toward fundamental or applied research, and that AR is the main threat to existing control strategies. Control of helminth infections in mainstream farming systems with fewer chemical inputs is a topical challenge and one that will require new research, technologies, and perhaps economic goals [1].

Questions around helminth epidemiology, management of AR, and alternative control approaches including *refugia*, were frequently repeated in the original list (see supplemental material), for example being posed more than once for different parasite or host taxa. To achieve feasible smaller research projects as envisaged at the start of this exercise, many of the questions could be broken back down again to specific taxa, both to produce system-

specific knowledge and applied solutions, and to explore the generality of conclusions from more studied contexts. Challenges in tropical or less developed countries yielded few specific questions, as did those related to pig and poultry production. Participation was strongly skewed towards European countries, in spite of efforts to be inclusive, possibly as a result of the European roots of LiHRA, under whose auspices the exercise was conducted (Box 1). Nevertheless, questions submitted from outside Europe focused on similar areas, and almost all of the final questions are relevant across wide geographic areas and often globally. The voting round (Box 2) might also have distorted results and led to the loss of original but less popular ideas from the final list, though such a step was necessary to limit numbers of questions and exclude some to which answers are already well-known. The full list is included as supplemental material to this article.

While not definitive, the final list of 100 questions serves to indicate current concerns among the livestock helminth research community, and highlights several areas in which existing understanding is poor while fresh advances now appear possible. The questions might serve to encourage or inspire work in those areas. For example, early career researchers might peruse the list to identify topics on which short or starter projects might have disproportionately high impact on the state of knowledge. It would be instructive to repeat this exercise in future, to determine how many of the questions have been answered, and whether the state of knowledge, the enabling technologies, or the problems of the day have moved sufficiently to generate different gaps and priorities. In the meantime, as a community, there is clearly work to be done to explore interesting questions whose answers are highly relevant to the ability of humankind to feed itself in the future while respecting the global environment and the health and welfare of the animals that sustain us.

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References

- [1] Vercruysse J. et al. (2018) Control of helminth ruminant infections by 2030. Parasitology doi: 10.1017/S003118201700227X
- [2] Kaplan, R.M. and Vidyashankar, A.N. (2012) An inconvenient truth: global worming and anthelmintic resistance. Vet. Parasitol. 186, 70-78
- [3] Rose, H. et al. (2015) Widespread anthelmintic resistance in European farmed ruminants: a systematic review. Vet. Rec. 176, 546
- [4] Sangster, N.C. et al. (2018) Ten events that defined anthelmintic resistance research. Trends Parasitol. DOI: <https://doi.org/10.1016/j.pt.2018.05.001>

764 [5] Van Dijk, J. et al. (2008) Climate change and infectious disease: helminthological
765 challenges to farmed ruminants in temperate regions. *Animal* 4, 377-392

766 [6] Charlier, J., et al. (2014). Practices to optimise gastrointestinal nematode control on
767 sheep, goat and cattle farms in Europe using targeted (selective) treatments. *Vet. Rec.* 175,
768 250

769 [7] Beesley, N.J. et al. (2018). *Fasciola* and fasciolosis in ruminants in Europe: Identifying
770 research needs. *Transbound. Emerg. Dis.* 256, 199-216

771 [8] Charlier, J. et. al. (2018) Mind the gaps in research on gastrointestinal nematodes of
772 veterinary importance. *Transbound. Emerg. Dis.* 65, 217-234

773 [9] Van Wyk, J.A. et al. (2011) Blueprint for an automated specific decision support system
774 for countering anthelmintic resistance in *Haemonchus* spp. at farm level. *Vet. Parasitol.* 177,
775 212-223

776 [10] Sutherland, W.J. et al. (2013) Identification of 100 fundamental ecological questions. *J.*
777 *Ecol.* 101, 58-67

778 [11] Gibbs, H.C. (1986) Hypobiosis in parasitic nematodes - an update. *Adv. Parasitol.* 25,
779 129-174

780 [12] Langrova, I. et al. (2008). Arrested development of sheep strongyles: onset and
781 resumption under field conditions of Central Europe. *Parasitol. Res.* 103, 387-392

782 [13] Cantacessi, C. et al. (2010) Deep insights into *Dictyocaulus viviparus* transcriptomes
783 provides unique prospects for new drug targets and disease intervention. *Biotechnol Adv.* 29:
784 10.1016/j.biotechadv.2010.11.005

785 [14] Rowe, A. et.al. (2008) *Haemonchus contortus* infection in sheep: Parasite fecundity
786 correlates with worm size and host lymphocyte counts. *Vet. Parasitol.* 153, 285-293

787 [15] Walker, J.G. and Morgan, E.R. (2014) Generalists at the interface: Nematode transition
788 between wild and domestic ungulates. *Int. J. Parasitol. Parasites Wildl.* 3, 242–250

789 [16] Armour, J. et al. (1988). Clinical nematodiriasis in calves due to *Nematodirus battus*
790 infection. Vet. Rec. 123, 230-231

791 [17] Pyziel, A.M. et al. (2016) Interrelationships of *Dictyocaulus* spp. In wild ruminants with
792 morphological description of *Dictyocaulus cervi* N. sp. (Nematoda: Trichostrongyloidea)
793 from red deer, *Cervus elaphus*. J. Parasitol. 103, 506-518

794 [18] Walker, J.G. et. al. (2018) Prediction and attenuation of seasonal spill over of parasites
795 between wild and domestic ungulates in an arid mixed-use system. J. Appl. Ecol. 55, 1976-
796 1986

797 [19] Chintoan-Uta, c. et al. (2014) Wild deer as potential vectors of anthelmintic-resistant
798 abomasal nematodes between cattle and sheep farms. Proc. R. Soc. B. Biol. Sci. 281,
799 20132985

800 [20] Cable, J. et al. (2017) Global change, parasite transmission and disease control: lessons
801 from ecology. Phil. Trans. R. Soc. B 372, 20160088

802 [21] Gethings, O.J. et al. (2015) Asynchrony in host and parasite phenology may decrease
803 disease risk in livestock under climate warming: *Nematodirus battus* in lambs as a case study.
804 Parasitology 142, 1306–1317

805 [22] Koelle, K. et al. (2005) Pathogen adaptation to seasonal forcing and climate change.
806 Proc. R. Soc. B 272, 971–977

807 [23] Huson, K. et al. (2017) Paramphistomosis of ruminants: an emerging parasitic disease in
808 Europe. Trends Parasitol. 33, 836-844

809 [24] Escarcha, J. et al. (2018) Livestock under climate change: a systematic review of
810 impacts and adaptation. Climate. 6. Dpi: 10.3390/cli6030054

811 [25] Molento, M. et al. (2016) Pasture larval count as a supporting method for parasite
812 epidemiology, population dynamic and control in ruminants. Livest. Sci. 192, 48-54

813 [26] Charlier J. et. al. (2015) ECONOHEALTH: placing helminth infections of livestock in
814 an economic and social context. *Vet. Parasitol.* 212, 62–67

815 [27] Kenyon, F. et al. (2013) Reduction in greenhouse gas emissions associated with worm
816 control in lambs. *Agriculture* 3, 271–284

817 [28] Houdijk, J.G.M. et al. (2011) Animal health and greenhouse gas intensity: the paradox of
818 periparturient parasitism. *Int. J. Parasitol.* 47, 633-641

819 [29] Williams, A.R. et al. (2015) A systems-life cycle assessment approach to modelling the
820 impact of improvements in cattle health on greenhouse gas emissions. *Adv. Anim. Biosci.* 6,
821 29–31

822 [30] Cooke, A.S. et al. (2017) Modelling the impact of targeted anthelmintic treatment of
823 cattle on dung fauna. *Environ. Toxicol. Pharmacol.* 55, 94-98

824 [31] Verdu, J.R. et al. (2018) Ivermectin residues disrupt dung beetle diversity, soil properties
825 and ecosystem functioning: An interdisciplinary field study. *Sci. Total Environ.* 618, 219-228

826 [32] Herrero, M. et al. (2015) Livestock and the environment: What have we learned in the
827 past decade? *Annu. Rev. Environ. Resour.* 40, 177-202

828 [33] Perry, B.D. and Randolph, T.F. (1999) Improving the assessment of the economic
829 impact of parasitic diseases and of their control in production animals, *Vet. Parasitol.* 84,
830 145-168

831 [34] Forbes, A.B., et al. (2000) Evaluation of the effects of nematode parasitism on grazing
832 behaviour, herbage intake and growth in young grazing cattle. *Vet. Parasitol.*, 90, 111-118

833 [35] Moore, J. (2002) Parasites and the behavior of animals. *Oxford Series in Ecology and*
834 *Evolution*. ISBN: 0–19–508441–1

835 [36] Szyszka, O. et al. (2013) Do the changes in the behaviours of cattle during parasitism
836 with *Ostertagia ostertagi* have a potential diagnostic value? *Vet. Parasitol.* 193, 214-222

837 [37] Hutchings, M.R. et al. (1998) Behavioural strategies used by parasitized and non-

838 parasitized sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces.
 839 Anim. Sci. 67, 97-106
 840 [38] Hoste H. et al. (2010) Goat-nematode interactions: think differently. Trends Parasitol.
 841 26, 376-381
 842 [39] Giacomini, P. et al. (2015) Suppression of inflammation by helminths: a role for the gut
 843 microbiota? Phil. Trans. R. Soc. B. Biol. Sci. 370, 1675, 0140296
 844 [40] Grenfell, B.T. (1988) Gastrointestinal nematode parasites and the stability and
 845 productivity of intensive ruminant grazing systems. Phil. Trans. R. Soc. Lond. B. Biol. Sci.
 846 321, 541-563
 847 [41] Garza-Cuartero, L. Et al. (2014) The worm turns: trematodes steering the course of co-
 848 infections. Vet. Path. 51, 385-392
 849 [42] Gause, W.C., and Maizels, R.M. (2016) Macrobionta-helminths as active participants and
 850 partners of the microbiota in host intestinal homeostasis. Curr. Opin. Microbiol. 32, 14-18
 851 [43] Midha, A. et al. (2017) Reciprocal interactions between nematodes and their microbial
 852 environments. Front. Cell. Infect. Microbiol. 7, 1-20
 853 [44] Peachey, L.E. et al. (2017) This gut ain't big enough for the both of us. Or is it?
 854 Helminth-microbiota interactions in veterinary species. Trends Parasitol. 33, 619-632
 855 [45] Steenhard, N.R. et al. (2009). *Ascaris suum* infection negatively affects the response to a
 856 *Mycoplasma hyopneumoniae* vaccination and subsequent challenge in pigs. Vaccine, 27,
 857 5161-5169
 858 [46] Andreasen, A. et al. (2015) Immune and inflammatory responses in pigs infected with
 859 *Trichuris suis* and *Oesophagostomum dentatum*. Vet. Parasitol. 207, 249–258
 860 [47] Ezenwa, V.O. (2016) Helminth-microparasite co-infection in wildlife: lessons from
 861 ruminants, rodents and rabbits. Parasit. Immunol. 38, 527-534

862 [48] Peachey, L.E. et al. (2018) The relationships between faecal egg counts and gut
863 microbial composition in UK Thoroughbreds infected by cyathostomins. *Int. J. Parasitol.*
864 Doi: 10.1016/j.ijpara.2017.11.003

865 [49] Clark et al 2018. Strongyle infection and gut microbiota: profiling of resistant and
866 susceptible horses over a grazing season. *Front. Physiol.* 9, 272

867 [50] Claridge J. et al. (2012) *Fasciola hepatica* is associated with the failure to detect bovine
868 tuberculosis in dairy cattle. *Nat. Commun.* 3, 853

869 [51] Hutchings, M. et al. (2007) Genetically resistant sheep avoid parasites to a greater extent
870 than do susceptible sheep. *Proc. R. Soc. Lond. B Biol. Sci.* 274, 1839-1844

871 [52] Greer, A.W. et al. (2018) Immune development and performance characteristics of
872 Romney sheep selected for either resistance or resilience to gastrointestinal nematodes. *Vet.*
873 *Parasitol.* 250, 60-67

874 [53] Riley, D.G. and Van Wyk, J.A. (2009) Genetic parameters for FAMACHA (c) score and
875 related traits for host resistance/resilience and production at differing severities of worm
876 challenge in a Merino flock in South Africa. *Vet. Parasitol.* 164, 44-52

877 [54] Bisset S.A. et al. (1994) Genetics of resilience to nematode parasites in Romney sheep.
878 *N.Z. J. Agric. Res.* 37, 521-534

879 [55] Greer, A.W. et al (2013) Evaluation of lambs subjected to a targeted selective treatment
880 anthelmintic regime. *Proc. N.Z. Soc. Anim. Prod.* 73, 175-179

881 [56] Raberg, L. Et al. (2009) Decomposing health: tolerance and resistance to parasites in
882 animals. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 37–49

883 [57] Wolstenholme, A.J. et al. (2004) Drug resistance in veterinary helminths. *Trends*
884 *Parasitol.* 20, 469-476

885 [58] Knapp-Lawitzke, F. et al. (2015) Rapid selection for β -tubulin alleles in codon 200
886 conferring benzimidazole resistance in an *Ostertagia ostertagi* isolate on pasture. Vet.
887 Parasitol. 209, 84-92

888 [59] Tarbiat, B. et al. (2017) Evaluation of benzimidazole resistance status in *Ascaridia galli*.
889 Parasitology 144, 1338-1345

890 [60] Rinaldi, L. et al. (2014) Comparison of individual and pooled faecal samples in sheep for
891 the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy
892 using McMaster and Mini-FLOTAC. Vet. Parasitol. 205, 216-223

893 [61] Kenyon, F. et al. (2016) Pooling sheep faecal samples for the assessment of anthelmintic
894 drug efficacy using McMaster and Mini-FLOTAC in gastrointestinal strongyle and
895 *Nematodirus* infection. Vet. Parasitol. 225, 53-60

896 [62] George, M. et al. (2017) Utilization of composite fecal samples for detection of
897 anthelmintic resistance in gastrointestinal nematodes of cattle. Vet. Parasitol. 240, 24-29

898 [63] Wyk, J.A. van (1997) Quality control in generic anthelmintics: Is it adequate? Vet.
899 Parasitol., 72, 157-165

900 [64] Learmount, J., et al. (2012). A computer simulation study to evaluate resistance
901 development with a derquantel–abamectin combination on UK sheep farms. Vet. Parasitol.
902 187, 244-253

903 [65] Leathwick, D. M. et al. (2015) Evidence for reversion towards anthelmintic
904 susceptibility in *Teladorsagia circumcincta* in response to resistance management
905 programmes. Int. J. Parasitol. Drugs Drug Resist. 5, 9-15

906 [66] Wyk, J.A. van (2001) Refugia - overlooked as perhaps the most potent factor concerning
907 the development of anthelmintic resistance. Onderstepoort J. Vet. Res. 68, 55-67

908 [67] Kenyon, F. et al. (2009) The role of targeted selective treatments in the development of
 909 refugia-based approaches to the control of gastrointestinal nematodes of small
 910 ruminants. *Vet. Parasitol.* 164, 3-11

911 [68] Cornelius, M.P. et al. (2016) Computer modelling of anthelmintic resistance and worm
 912 control outcomes for refugia-based nematode control strategies in Merino ewes in Western
 913 Australia. *Vet. Parasitol.* 220, 59-66

914 [69] Kenyon, F. et al. (2013) A comparative study of the effects of four treatment regimes on
 915 ivermectin efficacy, body weight and pasture contamination in lambs naturally infected with
 916 gastrointestinal nematodes in Scotland. *Int. J. Parasitol. Drugs Drug Res.* 3, 77-84

917 [70] Muchiut M. et al. (2018) Anthelmintic resistance: Management of parasite refugia for
 918 *Haemonchus contortus* through the replacement of resistant with susceptible populations.
 919 *Vet. Parasitol.* 254, 43-48

920 [71] Matthews J.B. et al. (2016) Progress in the development of subunit vaccines for
 921 gastrointestinal nematodes of ruminants: tools and technologies. *Parasite Immunol.* 38, 744-
 922 753

923 [72] Mahanty, S. et.al. (2017) TNF- α blockade suppresses pericystic inflammation following
 924 anthelmintic treatment in porcine neurocysticercosis. *PLoS Negl. Trop. Dis.* 11, e0006059

925 [73] Vlaminck J. et al. (2015) Vaccination of calves against *Cooperia oncophora* with a
 926 double-domain activation-associated secreted protein reduces parasite egg output and pasture
 927 contamination. *Int. J. Parasitol.* 45, 209-213

928 [74] Claerebout, E. et al. (2003) Current research and future prospects in the development of
 929 vaccines against gastrointestinal nematodes in cattle. *Exp. Rev. Vaccines* 2, 147-157

930 [75] Rose, H. et al. (2015) GLOWORM-FL: A simulation model of the effects of climate and
 931 climate change on the free-living stages of gastro-intestinal nematode parasites of ruminants.
 932 *Ecol. Model.* 297, 232-245

933 [76] Turner, J. et al. (2016) A model to assess the efficacy of vaccines for control of liver
 934 fluke infection. *Sci. Rep.* 6, 23345

935 [77] Sauermann, C.W. and Leathwick, D.M. (2018) A climate-driven model for the dynamics
 936 of the free-living stages of *Cooperia oncophora*. *Vet. Parasitol.* 255, 83-90

937 [78] Beltrame, L. et al. (accepted) A mechanistic hydro-epidemiological model of liver fluke
 938 risk. *J. R. Soc. Interface*

939 [79] Verschave, S.H. et al. (2016) Cattle and nematodes under global change: transmission
 940 models as an ally. *Trends Parasitol.* 32, 724-738

941 [80] Hoste, H. et al. (2015) Tannin containing legumes as a model for nutraceuticals against
 942 digestive parasites in livestock. *Vet. Parasitol.* 212, 5-17

943 [81] Ropiak, H.M. et al. (2016) Condensed tannins in extracts from European medicinal
 944 plants and herbal products. *J. Pharma. Biomed. Anal.* 120, 225-231

945 [82] Hoste, H. et al. (2012) Direct and indirect effects of bioactive legume forages against
 946 parasitic infections: experiences with tropical and temperate forages. *Vet. Parasitol.* 186, 18-
 947 27

948 [83] Williams, A.R. et al. (2014) Direct anthelmintic effects of condensed tannins from
 949 diverse plant sources against *Ascaris suum* PLoS One 9, e97053

950 [84] Williams, A.R. et al. (2017) Dietary cinnamaldehyde enhances acquisition of specific
 951 antibodies following helminth infection in pigs. *Vet. Immunol. Immunopathol.* 189, 43-52

952 [85] Gaudin, E. et al. (2016) Efficacy of sainfoin (*Onobrychis viciifolia*) pellet against multi
 953 resistant *H. contortus* and interaction with oral ivermectin: implication for on-farm control.
 954 *Vet. Parasitol.* 227, 122-129

955 [86] Šimpraga, M. et al. (2015) Alternative approaches for the control of gastrointestinal
 956 nematodes in sheep farming: a review. *Berl. Munch. Tierarztl. Wochenschr.* 128, 257-7070

957 [87] Takeuchi-Storm et al. (2017) Farm-level risk factors for *Fasciola hepatica* infection in
 958 Danish dairy cattle as evaluated by two diagnostic methods. *Parasit. Vectors*, v10, 555

959 [88] Howell et al. (1999) Control of gastrointestinal parasite larvae of ruminant using
 960 nitrogen fertilizer, limestone and sodium hypochlorite solutions. *Small Rumin. Res.* 32, 197-
 961 204

962 [89] Cairns et al. (2017) Potential anthelmintic properties of urea. *Proc. New Zealand Soc.*
 963 *Anim. Prod.* 77, 110-113

964 [90] Marley et al. (2003) The effect of dietary forage on the development and survival of
 965 helminth parasites in ovine faeces. *Vet. Parasitol.*, 93-107

966 [91] Ahmed, M.A. et al. (2013) Studies on the ability of two isolates of *Bacillus*
 967 *thuringiensis*, an isolate of *Clonostachys rosea* f. *rosea* and a diatomaceous earth product to
 968 control gastrointestinal nematodes of sheep. *Biocontrol Sci. Technol.* 23, 1067-1082

969 [92] Da Silveira, W.F. et al. (2017) Nematophagous fungi combinations reduce free-living
 970 stages of sheep gastrointestinal nematodes in the field. *J. Invert. Pathol.* 150, 1-5

971 [93] Charlier et al. (2016). Decision making on helminths in cattle: diagnostics, economics
 972 and human behaviour. *Ir. Vet. J.* 69:14

973 [94] Woodgate, R.G. and Love, S. (2012) WormKill to WormBoss: can we sell sustainable
 974 sheep worm control? *Vet. Parasitol.* 186, 51-57

975 [95] Van Wyk, J.A. (2008) Production trials involving use of the FAMACHA (c) system for
 976 haemonchosis in sheep: preliminary results. *Onderstepoort J. Vet. Res.* 75, 331-345

977 [96] Learmount, J. et al. (2018) Resistance delaying strategies on UK sheep farms: A cost
 978 benefit analysis. *Vet. Parasitol.* 254, 64-71

979 [97] Van de Velde et al. (2015) Diagnosis before treatment: Identifying dairy farmers'
 980 determinants for the adoption of sustainable practices in gastrointestinal nematode control.
 981 *Vet. Parasitol.* 212, 308-317

982 [98] Jack et al. (2017) A quantitative analysis of attitudes and behaviours concerning
983 sustainable parasite control practices from Scottish sheep farmers. *Prev. Vet. Med.* 139, 134-
984 145

985 [99] Van de Velde, F. et al. (2018) Beliefs, intentions, and beyond: A qualitative study on the
986 adoption of sustainable gastrointestinal nematode control practices in Flanders' dairy
987 industry. *Prev. Vet. Med.* 153, 15-23

988 [100] Van de Velde et al. (2018) Changing farmers' behavior intention with a hint of wit: The
989 moderating influence of humor on message sidedness. *Environ. Psychol.* 56, 97-103

990 [101] Ritter, C. et al. (2017) Determinants of farmers' adoption of management-based
991 strategies for infectious disease prevention and control. *J. Dairy Sci.* 100, 3329-3347

992 [102] Kristensen, E. and Jakobsen, E.B. (2011) Challenging the myth of the irrational dairy
993 farmer; understanding decision-making related to herd health. *N. Z. Vet. J.* 59, 1-7

994 [103] Charlier J. et al. (2014) Recent advances in the diagnosis, impact on production and
995 prediction of *Fasciola hepatica* in cattle. *Parasitology* 141, 326-335

996 [104] O'Brien, D. et al. (2017) DISCONTTOOLS: a database to identify research gaps on
997 vaccines, pharmaceuticals and diagnostics for the control of infectious diseases of animals.
998 *BMC Vet. Res.* 13, 1
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.

DISCONTTOOLS (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 DISCONTTOOLS 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

1025 competitive livestock sector in Europe. It represents key stakeholders from industry, farmers
1026 and research from across Europe. It is a knowledge-based lobby organisation working at the
1027 forefront of livestock related issues in Europe, including but not limited to animal health
1028 issues. The ATF unites members from every aspect of the livestock value chain (from feeding
1029 and breeding to production and processing), enabling an integrated approach to contribute to
1030 the environmental and societal challenges of livestock systems.

1031 The Livestock Helminth Research Alliance (LiHRA) (www.lihra.eu) is a consortium of
1032 researchers that aims to develop sustainable effective helminth control strategies and promote
1033 their implementation by the livestock industry. LiHRA grew out of EU-funded research
1034 projects addressing challenges in the control of gastrointestinal nematodes (FP6 PARASOL)
1035 and liver fluke (FP6 DELIVER) in ruminants under global change (FP7 GLOWORM), and
1036 related projects investigating alternative control approaches (Marie-Curie Initial Training
1037 Networks NematodeSystemHealth, Healthy Hay and Legume Plus, www.legumeplus.eu).

1038 LiHRA meets annually to review current challenges, recent results and opportunities for
1039 collaborative research. Discussions within LiHRA gave rise to the current article, and also
1040 underpinned the EU-funded networking COST Action COMBAR.

1041

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.

1067 The master list was reduced to 100 questions by online vote. Those who submitted questions,
1068 and the wider LiHRA membership, were asked to award each question zero, one, two or three
1069 stars, with more stars awarded to questions considered of high general importance and well
1070 suited to guide a focused and feasible research project or programme. The objective was to
1071 identify questions in important areas that are novel and testable, rather than those that are
1072 open-ended, general or already known. This choice was made using personal judgement, and
1073 there was no limit to the total number of stars that could be awarded by each voter. Question
1074 order was randomized for each participant. In total, 38 people voted, from a similar
1075 geographic profile as that of question submitters, comprising 15 countries, of which 11 in
1076 Europe, with many claiming direct experience of work in a wider range of locations spanning
1077 five continents.

1078 Questions were ranked according to total number of stars awarded, and in case of ties
1079 separated based on number of three-star scores awarded. When questions were repeated,
1080 effectively making the same point in a slightly different way, the highest scoring version was
1081 accepted, sometimes with minor changes to wording, others removed, and the next question
1082 on the list promoted into the top 100.

1083 A core group was constituted from those who engaged most vigorously with the process, and
1084 to cover the breadth of subject areas raised, as well as to bring perspectives from across the
1085 world. The core group made minor edits to questions, and then reached a consensus through
1086 written discussion on the split into ten topic areas, which represented major themes in the
1087 submitted list. The final list was presented in these sub-sections, with ranks removed.

1088 The methodology was adapted from earlier exercises in other subjects [10], modified to
1089 achieve greater global reach and less modification through repeated rounds of discussion. In
1090 this way, it was hoped that the final question list would capture a broad range of questions,
1091 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*.

DISCONTTOOLS – 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?

- 1218 28. How can we better practically detect and quantify viable liver fluke stages on pasture?
1219
- 1220 29. How can we better practically detect and species ID/profile GIN larvae on pasture?
1221 30. How can we define the key features of new anthelmintics, taking into account user and
1222 environmental safety?
1223 31. How can we effectively combine pasture management and parasite risk software?
1224 32. How do free living stages of nematodes adapt to climate change?
1225 33. How do infections with intestinal helminths affect the growth of young animals?
1226 34. How do parasitic worms respond to climatic change and what is the environmental
1227 plasticity?
1228 35. How do the different species of parasite present in an individual interact?
1229 36. How do water management and grazing practices interact to determine infection rate with
1230 *Schistosoma* species in ruminants?
1231 37. How does climatic change affect parasitism in grazing animals especially in semi-arid
1232 areas?
1233 38. How harmful are tapeworms to sheep and goats?
1234 39. How is climate change affecting overwintering of nematodes in temperate areas?
1235 40. How is hypobiosis from ruminant GIN terminated?
1236 41. How may massive anthelmintic chemotherapy in animal farming alter the life-traits of
1237 parasites?
1238 42. How to control helminthiasis among small ruminants?
1239 43. In co-grazing systems how often do cattle carry *Haemonchus contortus* and what are the
1240 consequences (biological and on weight gain or production)?
1241 44. Is *Dicrocoelium dendriticum* a parasite worth combatting?
1242 45. Is *Haemonchus* dominance really spreading in temperate areas and what difference
1243 should it make to worm control advice?
1244 46. Is the epidemiology of lungworm (*Dictyocaulus viviparus*) changing – why so many
1245 outbreaks in older (dairy) animals?
1246 47. Is the eradication of *Taenia solium* feasible?
1247 48. Is the recent prevalence increase of rumen fluke in Europe a threat to livestock farming?
1248
1249 49. Should we really aim to eliminate GIN in grazing animals or had we better sustain them?
1250
1251 50. To what extent are we dealing with neglected parasites when we are examining faecal
1252 samples?
1253 51. To what extent is extreme adaptation is considered genetic drift/shift in helminths?
1254 52. To what extent is there an exchange of parasites between wild and domestic ruminants?
1255
1256 53. What are the dynamics of resumption of development of inhibited larvae in horses
1257 (cyathostomes)?
1258 54. What are the emerging issues/diseases in helminthology?
1259 55. What are the functional roles of genomic ‘non-coding’ dark matter?
1260 56. What are the longitudinal infection dynamics of *Dictyocaulus viviparus* within a herd of
1261 supposedly immune cattle over a number of subsequent years?
1262 57. What are the major factors affecting infection levels of grazing animals with helminths?
1263
1264 58. What are the major genomic changes that enable species to adapt to a warmer climate?
1265
1266 59. What are the paramount parameters to assess the morbidity due to helminth infections?
1267

- 1268 60. What are valid grounds on which to separate parasite species?
1269 61. What do we understand about geographical differences and genetic variation in parasite
1270 populations?
1271 62. What is the balance between drift and selection in gastro-intestinal nematode evolution?
1272
1273 63. What is the cause of the reduction in voluntary feed intake in parasitized animals?
1274 64. What is the clinical relevance of AR in e.g. sheep or horses?
1275 65. What is the demonstrable effect of climate change on helminth parasites of livestock (+ve
1276 or -ve)?
1277 66. What is the difference in pathogenesis, effect on production, distribution and AR status
1278 between *Cooperia punctata*, *C. pectinata* and *C. oncophora*?
1279 67. What is the effect of helminth infection on GHG emissions from livestock, either directly
1280 or indirectly?
1281 68. What is the effect of weather/climate (especially drought) on the spatial distribution of
1282 GIN infective larvae on pasture and on the subsequent parasitological risk for grazing animals?
1283
1284 69. What is the efficient size of populations in gastrointestinal nematodes?
1285 70. What is the empirical evidence that different parasites will respond on global climate
1286 change?
1287 71. What is the epidemiology of *H. contortus* in northern Europe?
1288 72. What is the genetic basis behind hypobiosis?
1289 73. What is the impact of helminth parasitism in Europe in 2017?
1290 74. What is the influence of global change in the dynamics of the epidemiology of GIN?
1291 75. What is the inherent ability of a nematode to modulate its life-history traits to adapt to
1292 environmental pressures?
1293 76. What is the pathogenic effect of rumen fluke?
1294 77. What is the potential for parasite genomes? How should we use the information and what
1295 will they yield?
1296 78. What is the prevalence of various helminthoses?
1297 79. What is the relationship between parasitic diseases and the main infectious diseases of
1298 livestock?
1299 80. What is the relevance of the wild animal - domestic animal interphase for the main
1300 parasitic diseases of livestock?
1301 81. What is the role of wildlife in disseminating livestock parasites & AR
1302 82. What is the spatial distribution of helminth infections and how are they interrelated?
1303 83. What is the impact of anthelmintics on non-target fauna, functioning and ecosystem
1304 service provision?
1305 84. What percentage of adult dairy and beef cattle carry worms or lesions from *Ostertagia*
1306 and what effect does this have on production?
1307 85. When identifying wildlife reservoirs how much focus is put on identifying the direction
1308 of parasite transfer?
1309 86. Where did *Calicophoron daubneyi* come from?
1310 87. Which factors determine the length of the mucosal phase of *Ascaridia galli*?
1311 88. Which helminth is more affected by climate change? Is it temperate or tropical? Why?
1312
1313 89. Which parasites will be the winners and losers according to climate change models?
1314 90. Which user-friendly input data are required on a farm level to get useful output from a
1315 decision support tool or a transmission model?
1316 91. Why do horses lack important groupings of parasites that are common in other grazing
1317 ungulates?

- 1318 92. Will climate change result in a change of species in temperate environments or will the
 1319 existing ones simply adapt?
- 1320 93. What regulates egg production in females and can we suppress female egg production
 1321 sufficiently to provide an epidemiological advantage?
- 1322 94. Will breeding for resistance (low FECs and high production potential) drive nematode
 1323 adaptation towards increased fecundity to compensate?
 1324
- 1325 **Helminth biology and epidemiology - diagnostics**
- 1326 95. How can I see or detect that my flock or herd is infected by helminths?
- 1327 96. How can we improve the diagnosis of *Fasciola* spp?
- 1328 97. How far are we away from tests in the live animal for immature fluke and *Nematodirus*
 1329 infestations?
- 1330 98. How to predict a clinical case of dictyocaulosis in cattle?
- 1331 99. In a flock or herd, which sampling protocol should be followed for the diagnosis of
 1332 helminth infections?
- 1333 100. Is a mixed species of GINs in one animal difficult to control compared to an infected
 1334 animal with one GIN species?
- 1335 101. Is there some general European strategy for (manual) of examination of livestock for
 1336 helminthoses, before a treatment? Which methods are used in particular countries?
- 1337 102. What new technologies are used to detect infections by helminths in livestock?
- 1338 103. When will automated diagnostic tools/technologies be really available for on-farm
 1339 diagnosis?
- 1340 104. Which user-friendly parameters can help the farmer (or veterinarian) to make informed
 1341 decisions on helminth control in young stock?
- 1342 105. Why are faecal egg counts not at all times a good parameter to assess worm counts of
 1343 strongyles?
- 1344 **Economic and environmental impacts**
- 1345 106. From an economical and ecological point of view, what helminths do farmers think are
 1346 the most important? How would they list them?
- 1347 107. How accurately can we predict changes in the seasonality and magnitude of risk?
- 1348 108. How can helminth control be integrated in farm management in a cost-efficient way?
 1349
- 1350 109. How can we better assess production and health impacts of helminths?
- 1351 110. How can you measure environmental impacts of anthelmintics?
- 1352 111. Can we put an economic dollar value on the importance of a more strategic approach to
 1353 GIN treatment to producers?
- 1354 112. How does helminth control impact on the environment (MLs on microorganisms,
 1355 environmental schemes etc)?
- 1356 113. How important is it for us to chase subclinical GI nematodes in grazing beef cattle with
 1357 low FEC?
- 1358 114. How the three main farming systems (capitalistic, entrepreneur-type, peasant / small
 1359 farming / family farming) modify through values and technicity the parasite community?
- 1360 115. Is profitable livestock husbandry possible without chemical parasite control?
- 1361 116. Is there a market space to promote livestock products raised without (or with limited)
 1362 use of anthelmintics?
- 1363 117. Is there an association between countries or regions that have high levels of *Fasciola*
 1364 and level of income in those countries / regions?
- 1365 118. Is there an impact in the environment by the overuse of anthelmintics over the past
 1366 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?

- 1417 149. How do co-infections with helminths, and other infective organisms influence impact on
 1418 each other by direct or indirect immunologically related effects?
 1419 150. What is the role of co-infections e.g. bTB & fluke; ParaTB & GIN etc.?
 1420 151. What is the role of GIN in modifying the gut and lung microbiomes, and how does this
 1421 impact risk of bovine respiratory disease?
 1422 152. How do host-parasite relationships evolve when the initial conditions are nearly (but not
 1423 fully) the same: an application of the deterministic chaos of Poincaré?
 1424 153. How do GIN communicate in the GI tract?
 1425 154. How does interaction between different helminth species in co-infection affect the
 1426 immune system of the host?

1427 **Host resistance / resilience and selective breeding**

- 1429 155. Are there any advantages to being an individual that is prone to high parasite burdens?
 1430
 1431 156. Breeding for resilience (high FECs and high production potential) could result in
 1432 significantly increased pasture contamination over many years. What will the impact of
 1433 higher challenges be on resilient individuals? Will the resilience break down above a certain
 1434 threshold?
 1435 157. Can use of resilient sheep in a 'normal' flock (no *Haemonchus*) act as a source of
 1436 susceptible nematodes?
 1437 158. Has 60 years of intense anthelmintic use changed the relative susceptibility of livestock
 1438 to parasites? In other words, are animals wimpier than they used to be as a result of protection
 1439 from the effects of parasites by drugs, thereby causing selection of higher producing but more
 1440 parasite-susceptible animals?
 1441 159. How can genetic/gene manipulation be used in the parasite or the host to help with the
 1442 control of helminths?
 1443 160. To what extent is the impact of strongylid infections in ruminants dependent on host
 1444 resilience?
 1445 161. Under what circumstances should breeders aim for resilience, versus resistance, in
 1446 livestock?
 1447 162. What impact will breeding of sheep for resistance and resilience to nematodes have on
 1448 nematode challenge and adaptation?
 1449 163. Which are the main differences between cattle, sheep and goats in term of
 1450 resistance/susceptibility to helminth infection?
 1451 164. Which genotypes of livestock hold natural resistance to helminths?
 1452 165. What do we understand about the fitness cost of resistance and how can it be measured?
 1453 166. Why are some animals more prone to heavy parasite burdens than others?
 1454 167. How to measure and distinguish the resilience and the resistance of ruminants infected
 1455 with GIN?
 1456 168. Is resistance or tolerance a better breeding objective to produce small ruminants that
 1457 require less anthelmintic treatment?
 1458 169. Can targeted selective treatment, e.g. using FAMACHA, be used to select for parasite
 1459 resilience, especially among low-input traditional breeds?
 1460 170. In non-selective breeding systems, does TST support weak animals and lead to loss of
 1461 resilience at herd or flock level?
 1462 171. What are the life-time trade-offs between immunity to helminths and impacts on growth
 1463 and production, in different livestock systems?

1464 **Development and detection of anthelmintic resistance**

- 1466 172. Are data on drug failure/drug resistance within countries publicly available and are they
1467 reliable enough to be used as a mechanism to survey drug failure/resistance at a national /
1468 international level?
- 1469 173. Are data related to helminth resistance available for particular European countries?
- 1470 174. Can the use of combination drugs help to slow down the development of anthelmintic
1471 resistance?
- 1472 175. Can we develop markers for susceptibility to ML anthelmintics?
- 1473 176. Can we improve methods for monitoring efficacy of current control methods (e.g.
1474 surveillance, diagnostics and resistance testing)?
- 1475 177. Can we replace worm egg counts with an on-farm 'colour-change', e.g. ELISA,
1476 technology?
- 1477 178. Do combinatorial effects of different resistance mechanisms (i.e. target-associated and
1478 non-target-associated) exist and if so to what effect is this relevant in the field?
- 1479 179. Do differences in life history traits and reproductive strategy affect the risk for
1480 development of anthelmintic resistance?
- 1481 180. Do intra-ruminal bolus systems have an impact on the development of anthelmintic
1482 resistance?
- 1483 181. Does copy number variation have a role in anthelmintic resistance?
- 1484 182. Does gene duplication play a role in anthelmintic resistance?
- 1485 183. Does selection by ivermectin preselect for moxidectin resistance?
- 1486 184. Has the selection for drug resistance changed the pathogenicity of parasites?
- 1487 185. How can the knowledge on AR in livestock be used to promote a better understanding of
1488 the development and mechanisms of AR in human GIN?
- 1489 186. How can we design anthelmintic combinations that are more effective and that
1490 should/would limit resistance development?
- 1491 187. How can we develop molecular markers for ML drugs?
- 1492 188. How can we improve diagnostics: infection intensities and drug resistance?
- 1493 189. How do we prevent anthelmintic resistance, when change makes it a moving target?
- 1494 190. How does animal movement affect the spread of helminth infections and anthelmintic
1495 resistance?
- 1496 191. How fast is AR developing in cattle nematodes?
- 1497 192. How is size of refugia needed affected by the genetics of ML resistance?
- 1498 193. How predictive can be a gastro-intestinal nematode model in terms of resistance
1499 appearance and emergence?
- 1500 194. How useful are composite faecal egg counts to detect anthelmintic resistance?
- 1501 195. *In-vitro*/genetic/lab methods for detection of anthelmintic resistances: desirable,
1502 reachable and applicable for all anthelmintic drug groups?
- 1503 196. Is there evidence of selection for ML-R when treating for sheep scab?
- 1504 197. Is treatment of ectoparasites with macrocyclic lactone drugs an important driver of
1505 anthelmintic resistance in sheep?
- 1506 198. Practically, what should the percentage of sheep/goats/cows/heifers left untreated in a
1507 group to control the emergence of anthelmintic resistance?
- 1508 199. What are the best diagnostic techniques to detect anthelmintic resistance?
- 1509 200. What are the contributory factors for the development of anthelmintic resistance?
- 1510 201. What are the key factors involved in the development of AH resistance, and mitigation
1511 measures?
- 1512 202. What are the molecular mechanisms involved in resistance to macrocyclic lactones?
- 1513 203. What are the prospects for identifying molecular markers for resistance?
- 1514 204. What are the risk factors for multiple anthelmintic resistance development in cattle?

- 1515 205. What changes in genes other than the immediate drug target, such as transporters and
1516 drug metabolism are involved in drug resistance?
- 1517 206. What do genotype-phenotype studies tell us about the quantitative contribution of a
1518 particular mutation to the resistance phenotype?
- 1519 207. What do we learn from the virtual absence of anthelmintic resistance in cattle?
- 1520 208. What drugs are the cause of higher prevalence of anthelmintic resistance in cattle, sheep
1521 and goats?
- 1522 209. What factors are involved in the development of anthelmintic resistance?
- 1523 210. What factors drive the emergence of anthelmintic resistance?
- 1524 211. What is the best way for in vivo quantitative evaluation of GIN burden in cattle?
- 1525 212. What is the effect of long lasting moxidectin injections on the development of ML
1526 resistance in sheep and cattle?
- 1527 213. What is the empirical evidence for a lack of reversion to susceptibility when drug
1528 selection pressure is removed?
- 1529 214. What is the global scenario of prevalence and optimal methods for detection of
1530 anthelmintic resistance in ruminants?
- 1531 215. What is the key to molecular assays capable of detecting resistant worms?
- 1532 216. What is the link between genetic variation and the risk for selection of resistance?
- 1533 217. What is the relative importance of management versus environmental factors in
1534 determining the development of anthelmintic resistance in livestock?
- 1535 218. What is the role of combination i.e. dual-active anthelmintics in current helminth
1536 control?
- 1537 219. What is the role of sequencing (WGS/NGS) in understanding the genetic basis of AR in
1538 GIN & fluke?
- 1539 220. What is the status of drug resistance in *Ascaris suum* and other important pig parasites?
1540
- 1541 221. What is the true, non-biased, prevalence of anthelmintic resistance?
- 1542 222. What makes a parasite resistant to anthelmintics?
- 1543 223. What role does the individual animal play in the development of drug resistance in a
1544 parasite population?
- 1545 224. What specific genetic differences either cause resistance or are sufficiently closely
1546 associated with resistance to be able to serve as molecular markers?
- 1547 225. Where are we at present in anthelmintic resistance in farm animals?
- 1548 226. Which are the most rapid and accurate methods to detect the anthelmintic resistance?
1549
- 1550 227. Which are the newest anthelmintics available in the market, and is there any report about
1551 flock or herds resistant to these ones?
- 1552 228. Which genes are implicated in the development of anthelmintic resistance according to
1553 the family of anthelmintic?
- 1554 229. Why did AR (at least thus far) not occur in most gastro-intestinal helminths of dogs and
1555 cats?
- 1556 230. Why is it so difficult to identify markers for genetic resistance?
- 1557 231. Is there (genetic) evidence for reversion to susceptibility under any circumstances?
1558
- 1559 **Practical management of anthelmintic resistance**
- 1560 232. Anthelmintic treatment and control programmes: where, who, when and how?
- 1561 233. Are combination anthelmintics useful to combat anthelmintic resistance?
- 1562 234. Are current control programmes suitable for helminths in livestock considering all or
1563 most of the productivity systems?

1564 235. Can 'farmer's eye' be used effectively to slow the development of AR in sheep flocks (it
1565 works but what about its effect on performance)?

1566 236. Can we expect new anthelmintic compounds on the market in the (near) future?

1567 237. How much are the major pharmaceutical companies investing in new anthelmintics,
1568 specifically?

1569 238. We are on the cusp of having molecular markers for drug resistance e.g. macrocyclic
1570 lactone resistance in *Haemonchus contortus* and triclabendazole resistance in liver fluke.
1571 How should we best apply these markers?

1572 239. Should focus on new drug discovery ensure the target is just one class of parasite so that
1573 resistance development due to inadvertent use can be minimised? E.g. if an injectable
1574 treatment for external parasites such as scab can be developed which doesn't also control
1575 roundworms.

1576 240. What are the limitations for developing anthelmintic combinations?

1577 241. What are the prospects for a new flukicide to treat immature/acute infection, especially
1578 in sheep?

1579 242. What are the prospects for any novel anthelmintics, given experiences with new AADs
1580 & dual-actives?

1581 243. What is the value of faecal egg count monitoring as a decision tool in anthelmintic
1582 treatments?

1583 244. Is TST a feasible approach with which to control helminths with a very high biotic
1584 potential, e.g. the ascarids?

1585 245. What reporting systems are in place to record drug failure/drug resistance within
1586 countries?

1587 246. Could an anthelmintic-resistant flock or herd get back to be susceptible and how?

1588 247. Describe the methods of integrated helminth parasite control?

1589 248. Can we automate TST data interpretation, also for farmer training?

1590 249. How can flukicides be applied more effectively, is refugia an option?

1591 250. How can we make control more effective and sustainable?

1592 251. How do we apply existing knowledge of the risk factors for anthelmintic resistance on
1593 farms to effectively slow its development?

1594 252. How can we reverse AH resistance?

1595 253. How do we implement better dosing procedures of anthelmintics to cattle in order to
1596 insure therapeutic drug levels (pour-on vs. injection/oral)?

1597 254. How do we solve the conundrum of use of anthelmintic drug combinations – or when to
1598 use drug combinations and when not to?

1599 255. How does the level of refugia influence the emergence of resistant phenotypes?

1600 256. How to control anthelmintic resistance?

1601 257. Is anthelmintic resistance genuinely irreversible or can susceptibility be restored within
1602 helminth populations?

1603 258. Is deworming sheep or goats truly necessary?

1604 259. Under what circumstances are combination drugs the answer to manage anthelmintic
1605 resistance?

1606 260. What (empirical) evidence is there that refugia slows down the development of drug
1607 resistance?

1608 261. What are the best strategies to prevent further spread of anthelmintic resistance (in small
1609 ruminants)?

1610 262. What are the characteristics of an optimal quarantine drench as a way of reducing the
1611 risk of importing resistance with bought in animals?

1612 263. What is the efficacy of mitigation measures to reduce non-target impacts of anthelmintic
1613 on the environment?

- 1614 264. What is the optimal use of fasciolicides where there is triclabendazole resistance?
 1615 265. What is the role of refugia in slowing selection for AR in sheep/cattle GIN?
 1616 266. What is the usefulness of anthelmintics working at decreased (50% or 80%) efficacy?
 1617
 1618 267. What proportion of a parasite population must be left in refugia?
 1619 268. What steps should be taken when resistance to all known anthelmintic drug classes
 1620 develops?
 1621 269. Is refugia relevant for all parasite species; if not, what realistic alternatives exist for
 1622 those parasites that display drug resistance but for which refugia based control is not deemed
 1623 appropriate?
 1624 270. What will be the best methods to control *Fasciola* in areas where there is free grazing?
 1625
 1626 271. Why is development of anthelmintic resistance not reversible, even in the absence of the
 1627 specific drug?
 1628 272. Is targeted selective treatment sustainable in the long term?
 1629 273. Why is the (parasitological) community accepting strategic anthelmintic treatments
 1630 against GIN in cows (not learning from the small ruminant example)?
 1631 274. With good parasite management can on farm anthelmintic resistance be reversed?
 1632 Especially to 2LV and 3ML classes of drugs as has been found in NZ?
 1633 275. Is there a link between the size of the refugia needed to prevent AR and the molecule
 1634 used (persistent versus non persistent)?
 1635 276. How does the level of refugia influence the detection and spread of resistant phenotype
 1636 in different hosts, different parasites and different treatment systems?
 1637 277. Is there a role for refugia in control of liver fluke?
 1638 278. What are the most useful decision parameters in targeted selective anthelmintic
 1639 treatments?
 1640
 1641 **Vaccines and immunology**
 1642 279. Can we develop sustainable methods of control (eg. vaccines and management)?
 1643 280. Can we enhance the natural immune response to helminths by applying a biological
 1644 treatment (e.g. specific cytokine or cytokine inhibitor) and thereby control them effectively?
 1645
 1646 281. Could immune-stimulatory drugs for livestock be used for combating helminths?
 1647 282. Does *Fasciola* modulate co-infection with other parasites?
 1648 283. Do worms have a microbiome? Can it be exploited as a vaccine or treatment target?
 1649 284. How are optimal helminth vaccination schedules influenced by infection pressure and
 1650 can this be incorporated into decision making?
 1651 285. How can vaccines against helminth infections in ruminants be integrated in control
 1652 programmes?
 1653 286. How can we develop and apply vaccines?
 1654 287. How does the parasite resist or escape from the host immune system?
 1655 288. How fast do parasites adapt to increased immune selection pressures (due to for instance
 1656 vaccines)?
 1657 289. How may massive anthelmintic chemotherapy in animal farming alter host immunity
 1658 structuration?
 1659 290. How well do anti-helminth vaccines have to work to be useful?
 1660 291. How would vaccines against soil-transmitted helminth infections influence population
 1661 dynamics?
 1662 292. To what extent does overuse of/use of very effective anthelmintic products affect
 1663 development of immunity to bovine lungworm?

- 1664 293. To what extent is the immunomodulation by helminth parasites detrimental to the
 1665 animal's health when co-infections co-occur?
- 1666 294. What are the crucial effects that a vaccine against helminth(s) need to produce so that
 1667 farmers agree to include them in their farm management?
- 1668 295. What is the future for (recombinant) vaccines?
- 1669 296. What is the future of vaccines against helminths of livestock?
- 1670 297. What is the immunological difference between host species showing widely different
 1671 responses to closely related parasite species (eg. cattle versus donkey with respect to
 1672 *Dictyocaulus* spp.)?
- 1673 298. What is the potential for a multivalent vaccine to control multiple species?
- 1674 299. What is the potential for vaccines to control individual helminth species?
- 1675 300. What mechanisms are involved in protective immunity against helminths?
- 1676 301. What regulates egg production in females and can we suppress female egg production
 1677 sufficiently to provide an epidemiological advantage?
- 1678 302. Which efficacy is needed from a helminth vaccine and how can vaccination be
 1679 integrated in sustainable parasite control?
- 1680 303. Why don't we yet have vaccines to control helminth infections in livestock?
- 1681 304. Why is the efficacy of the *Haemonchus* vaccine (hidden antigen approach) much lower
 1682 in adult sheep?
- 1683 305. Why is the protective immunity to *Ascaridia galli* limited or almost absent?

1684 1685 **Alternative approaches to helminth management**

- 1686 306. Are there basic processes in egg hatching or larval development that can be manipulated
 1687 to aid control?
- 1688 307. Are there possible escaping mechanisms of GIN to alternative approaches (e.g. vaccines,
 1689 bioactive compounds)?
- 1690 308. As challenge increases, will this result in an increase in the proportion of the flock/herd
 1691 needing treatment over time?
- 1692 309. Can anthelmintic resistance be reversed through TST, good management or reseedling
 1693 approaches?
- 1694 310. Can different bioactive plants be combined to increase effects on GI nematodes?
- 1695 311. Can knowledge of risk factors for nematode infection in cattle, derived from antibody
 1696 testing, be used to target treatments more effectively within as well as between herds?
- 1697 312. Can TSTs be applied to cattle or pig parasites?
- 1698 313. Can we cultivate plants for grazing which have maximum nutritive value and the
 1699 potential to lower helminth burden?
- 1700 314. Can we manipulate the intermediate host (e.g. *Galba truncatula*) to help control
 1701 *Fasciola hepatica* and *Calicophoron daubneyi*?
- 1702 315. Can we use polyphenols or other natural compounds found in forage to control
 1703 helminths of livestock?
- 1704 316. Does a natural polyphenol causing 100% inhibition of L3 of GIN larvae *in vitro*
 1705 represent a promising natural compound for integrated helminths control??
- 1706 317. Does feeding of probiotics improve resistance to and outcome of GI helminth infection?
- 1707
- 1708 318. Does the inhibition of exsheathment of L3 stage of gastrointestinal nematodes represent
 1709 a viable control method for these helminths?
- 1710 319. How can investigation of tank milk be an attractive monitoring tool so that it can be used
 1711 as a basis for intervention strategies?
- 1712 320. How do we develop easy, on-farm tools (diagnosis) for the implementation of targeted
 1713 selected treatments?

- 1714 321. How does processing and conservation of bioactive forages affect their efficacy?
 1715 322. How is the pharmacokinetic behaviour of bioactive plant compounds in relation to
 1716 parasitic nematodes situated in different body compartments (i.e. small intestine, large
 1717 intestine, liver, lungs)?
 1718 323. How should vaccines be combined with anthelmintics to optimise control?
 1719 324. How successful are herbs as an alternative of anthelmintic to livestock helminth?
 1720 325. If reduced effectiveness of TST over time transpires, could targeted treatment instead of
 1721 TST be used to minimise pasture contamination at strategic intervals e.g. every few years at a
 1722 time of year when egg development success is greatest?
 1723 326. Is on-farm TST applicable in cattle viz-a-viz FAMACHA in sheep?
 1724 327. How can we practically target free-living gastrointestinal nematode stages outside the
 1725 host?
 1726 328. Is TT (treating at times of highest risk) inherently incompatible with the aim of
 1727 maximising refugia? E.g. by treating at the time when risk is highest (usually when
 1728 development success is high) we are increasing the selection pressure.
 1729 329. Many studies have shown a maximum efficacy of bioactive (plant) compounds around
 1730 60-70% reduction – how do we get a higher efficacy? Is it needed?
 1731 330. Should TST be adapted to overall infection levels, such that whole-herd treatments are
 1732 sometimes optimal?
 1733 331. To what extent should TST indicators for nematode infection be extended to include
 1734 arthropod parasites?
 1735 332. What are the alternatives to anthelmintic drugs?
 1736 333. What are the interactions between bioactive forages and synthetic anthelmintic drugs, *in*
 1737 *vitro* and *in vivo*?
 1738 334. How successful are herbs as an alternative of anthelmintic to livestock helminth?
 1739 335. What are the limitations of pasture management routines?
 1740 336. What are the mechanism of action of bioactive plant compounds and metabolites in
 1741 relation to parasite establishment and adult worms?
 1742 337. What is effective worm control within a context of sustainability?
 1743 338. What is the best alternative to anthelmintics?
 1744 339. What is the effect of the use of alternative control measures (i.e. bioactive plants) as
 1745 regards AH resistance?
 1746 340. What is the efficacy of alternative methods of livestock parasite control?
 1747 341. What is the efficacy of dung beetles for livestock helminth control?
 1748 342. What is the role of medicinal plants for developing new anthelmintics?
 1749 343. What should be the minimal size of a refugia population to ensure the efficacy of a TST
 1750 strategy to prevent AR in ruminants?
 1751 344. Why does the *Duddingtonia* (BC) approach work less well in small ruminants?
 1752 345. Will TST result in increased pasture contamination over many years? Especially with
 1753 increased overwinter survival of L3 on pasture.
 1754 346. What is the efficacy of plant based anthelmintics against drug resistant helminths?
 1755 347. What are the main obstacles to the development of new technologies to control
 1756 helminths of livestock?

1757 **Stakeholder engagement**

- 1759 348. Are farmers able to adapt or do they need support (e.g. from predictive models)? Does
 1760 this vary by sector e.g. dairy vs sheep?
 1761 349. Are farmers and/or vets from rural regions being well advised on what are the best
 1762 practices for parasite control in their area?
 1763 350. Are our models any better than farmers' intuition?

1764 351. Can veterinary surgeons get more involved in parasite control on sheep farms?
1765
1766 352. Can we convince producers to adopt more sustainable control practices (where
1767 resistance is not yet an issue; to prevent its development)?
1768 353. How can different novel control methods for GI nematodes be integrated effectively and
1769 in a way that is simple enough for farmers to implement?
1770 354. How can famer perceptions of anthelmintic resistance as something that happens to
1771 others be overcome to increase their efforts to combat it?
1772 355. How can we better promote best practices of diagnosis and treatment for helminth
1773 control in livestock?
1774 356. How can we improve uptake of sustainable parasite control measures by vets and
1775 farmers?
1776 357. How can we increase correct management against parasitoses by livestock farmers?
1777 358. How can we refine spatial granularity of farmers' data whilst protecting privacy?
1778 359. How do we (the vet parasitology research community) achieve recognition for scientific
1779 papers that are aimed at practitioners, who do not publish themselves and therefore add
1780 nothing to citation rates?
1781 360. Can we be more creative in delivering alternative control options to farmers, including
1782 in less developed countries?
1783 361. How do we communicate the importance of a more strategic approach to GIN treatment
1784 to producers? Can we put an economic dollar value on it?
1785 362. How does the attitude of farmers with respect to accepting and implementing parasite
1786 control measures differ between countries?
1787 363. How sustainability are farmer out-reach projects on helminths?
1788 364. How to improve the relationships (eg submission of shared projects) between Vet and
1789 Medical Helminthology (Parasitology)?
1790 365. How will consumers influence livestock production practices, in terms of anthelmintic
1791 use?
1792 366. How will farmers adapt to the impact of climate change (increased climate variability)
1793 on disease risk?
1794 367. If tools were available to support farmers, what is the best way to encourage their use?
1795 Demonstration farms etc.?
1796 368. In which direction can we improve evidence based medicine for helminth control by
1797 dairy veterinarians?
1798 369. Is research in veterinary helminth infections reaching livestock farmers in developing
1799 countries and, if so, what is the impact?
1800 370. Is the stronger regulation of the sale of anthelmintics the only current way to slow the
1801 continued development of anthelmintic resistance?
1802 371. Vets, farmers, pharmaceuticals, researchers, stakeholders: which role for each one in the
1803 integrated control of parasites?
1804 372. What are the treatment approaches currently applied by producers?
1805 373. What factors drive anthelmintic treatment decisions by farmers?
1806 374. What is the optimal way to deliver spatial decision support to farmers?
1807 375. What is the role of human behaviour and psychology on livestock diseases?
1808 376. What kind of practice from the farmer would help to get livestock free of helminths?
1809 377. Why do most trust more on chemical parasite control than on adapting animal husbandry
1810 and grazing based on parasite life cycles?
1811 378. Why does farmer uptake of crucially important recommendations fail?
1812 379. Why we have been failing to achieve an integrated and sustainable helminth control
1813 programme?

- 1814 380. Can we integrate helminth control decision support tools in farm management software?
1815 381. How can we transfer automated technology to farmers, especially those that are
1816 resource-poor?
1817 382. What can we learn from social sciences to transfer knowledge on helminth control to
1818 farmers?

1819

1820 **Others**

- 1821 383. How can we best protect parasitology as a distinct discipline in ‘systems-based’
1822 veterinary school curricula?
1823 384. How do helminths infections in livestock impact stunting rates in children of subsistence
1824 farmers?
1825 385. What is a helminth parasite?
1826 386. What is the better way to fight these pests?
1827 387. What is the effect of parasite control programmes on product quality and safety?
1828 388. What is the European general treatment strategy of treatment of helminths in livestock?
1829 Which chemotherapeutics are used in particular countries?