100 Questions in Livestock Helminthology Research

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Eric R. Morgan[1]*, Nor-Azlina A. Aziz[2], Alexandra Blanchard[3], Johannes Charlier[4],

Claude Charvet[5], Edwin Claerebout[6], Peter Geldhof[6], Andrew W. Greer[7], Hubertus

Hertzberg[8], Jane Hodgkinson[9], Johan Höglund[10], Hervé Hoste[11], Ray M. Kaplan[12]

María Martínez Valladares[13], Siân Mitchell[14], Harm W. Ploeger[15], Laura Rinaldi[16],

Georg von Samson-Himmelstjerna[17], Smaragda Sotiraki[18], Manuela Schnyder[8], Philip

Skuce[19], David Bartley[19], Fiona Kenyon[19], Stig M. Thamsborg[20], Hannah Rose Vineer[21],

Theo de Waal[22], Andrew R. Williams[20], Jan A. van Wyk[23], Jozef Vercruysse[6]

1. Queen's University Belfast, School of Biological Sciences, 97, Lisburn Road, Belfast, BT9
   7BL, Northern Ireland, United Kingdom.

2. Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine,
   Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

3. Pancosma, voie des traz 6, CH-1218 Le Grand Saconnex (Geneva), Switzerland.

4. Kreavet, Hendrik Mertensstraat 17, 9150 Kruibeke, Belgium.

5. ISP, INRA, Université Tours, UMR1282, 37380, Nouzilly, France.

6. Laboratory for Parasitology, Faculty of Veterinary Medicine, Ghent University, B9820
   Merelbeke, Belgium.

7. Faculty of Agriculture and Life Sciences, P.O. Box 85084, Lincoln University,
   Christchurch, 7647, New Zealand.

8. Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, 8057 Zurich,
   Switzerland.

9. Institute of Infection and Global Health, University of Liverpool, Liverpool Science Park
   IC2, 146 Brownlow Hill, Liverpool, L3 5RF, UK

25 10. Swedish University of Agricultural Sciences, BVF-parasitology, Box 7036, 750 07,

26   Uppsala, Sweden.

27 11. UMR 1225 IHAP INRA/ENVT, 23 Chemin des Capelles, 31076 Toulouse, France.

28 12. Department of Infectious Diseases, College of Veterinary Medicine, University of

29   Georgia, Athens, Georgia, USA.

30 13. Instituto de Ganadería de Montaña (CSIC-Universidad de León). Finca Marzanas,

31   Grulleros, 24346 León, Spain.

32 14. Animal and Plant Health Agency, Carmarthen Veterinary Investigation Centre, Jobswell

33   Rd, Johnstown, Carmarthen, SA31 3EZ, Wales, United Kingdom.

34 15. Utrecht University, Department of Infectious Diseases and Immunology, Yalelaan 1,

35   3584 CL, Utrecht, The Netherlands.

36 16. Department of Veterinary Medicine and Animal Production, University of Napoli

37   Federico II, Napoli, Italy.

38 17. Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitaet Berlin,

39   Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Germany.

40 18. Veterinary Research Institute, HAO-DEMETER, Campus Thermi 57001 Thessaloniki

41   Greece.

42 19. Moredun Research Institute, Pentlands Science Park, Edinburgh EH26 0PZ, Scotland,

43   United Kingdom.

44 20. Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences,

45   University of Copenhagen, Frederiksberg, Denmark.

46 21. School of Biological Sciences, University of Bristol, 24 Tyndall Avenue, Bristol, BS8

47   1TQ, United Kingdom.

48 22. University College Dublin, School of Veterinary Medicine, Belfield, Dublin, D04 W6F6,

49   Ireland.

50   23. Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X20,

51   Pretoria, South Africa.

52   *Correspondence: eric.morgan@qub.ac.uk

53

Abstract

55   An elicitation exercise was conducted to collect and identify pressing questions concerning

56   the study of helminths in livestock, to help guide research priorities. Questions were invited

57   from the research community in an inclusive way. Of 385 questions submitted, 100 were

58   chosen by online vote, with priority given to open questions in important areas that are

59   specific enough to permit investigation within a focused project or programme of research.

60   The final list of questions was divided into ten themes. We present the questions and set them

61   briefly in the context of the current state of knowledge. Although subjective, results provide a

62   snapshot of current concerns and perceived priorities in the field of livestock helminthology,

63   and we hope will stimulate ongoing or new research efforts.

Key words:

66

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Introduction: towards inclusive identification of research priorities

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

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

92   feed into priority-setting by funders and policy makers, as well as produce published outputs

93   [7,8]. In other cases, experts produce opinionated reviews on the state of the art and expound

94   a vision of the way forward [1,4,9]. These exercises are built on consensus, often among

95   those who have worked together over a sustained period to develop ideas and drive progress

96   in the field. While these approaches are undoubtedly useful, they tend to perpetuate dominant

97   current thinking, and potentially neglect marginal but promising suggestions.

98   Alternatives are possible. Inspired by previous attempts in ecology [10], we here consult

99   more widely across the research community to identify key current questions in livestock

100   helminthology, to motivate and guide new work. The number 100 was chosen such that

101   questions might be broad enough to be strategically important, yet focused enough to be

102   tackled within a single focused research project or programme [10]. We elicited questions

103   from as wide a base as possible within the discipline (Box 2), to reduce the influence of

104   expert views and established dogmas on the questions presented, and to allow for disruptive

105   and creative ideas. Further rounds of voting and organization followed, and here we list the

106   questions judged most meritorious by a broad panel of specialists. The ten sub-sections are

107   based on the questions received and were not decided beforehand, and text commentary

108   follows rather than precedes each series of questions, in keeping with the 'bottom-up' spirit

109   of the exercise. The sections are structured to progress in a general direction from processes

110   of infection, through impacts, to control through chemical and alternative means, and include

111   challenges across the spectrum of fundamental and applied research. While we make no

112   claim to this list being definitive or complete, it is a snapshot of what researchers in livestock

113   helminthology consider to be important and topical at this time, and we hope that it will

114   stimulate discussion, and renew energy in existing or novel directions.

115

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

Hypobiosis

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

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

Fecundity

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

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

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

6

142   worm size is highly correlated with the number of eggs present in adult females, and egg

143   production is limited by host immune regulation [15]. Ability to target fecundity specifically,

144   and evolutionary responses of parasites to such a strategy, are therefore likely to be highly

145   dependent on other parasite traits as well as host factors.

146

147   *Parasite adaptation to new hosts*

148   4. To what extent is there an exchange of parasites between wild and domestic ruminants?

149   5. Does cross-grazing of cattle and small ruminants encourage gastrointestinal nematode

150   species to adapt and cross between hosts?

151

152   Gastrointestinal nematode (GIN) species tend to have a preferred host, but there is

153   considerable evidence to indicate transmission and adaptation between livestock species

154   (sheep/goat/cattle) and between livestock and wildlife when either co-grazed or grazed

155   alternately on the same pasture [15]. In farming systems, control by means of alternate

156   grazing with different host species has been reported to break down due to parasite adaptation

157   [16]. Older studies often lack genotyping and apparent infection across multiple host species

158   may therefore constitute different parasite subpopulations or even species with cryptic host

159   preferences, as with lungworms in deer [17]. Whether the impact of cross-transmission

160   between wildlife and livestock is likely to amplify or reduce pasture infectivity and thus

161   transmission to livestock is in general an open question and likely to be context-specific [18].

162   Untreated wildlife could, moreover, act as a source of *refugia* for drug-susceptible genotypes,

163   or alternatively transfer resistant parasites to new hosts or locations [19]. The net effect of

164   livestock-wildlife contact on helminth ecology and evolution is hard to predict.

165

166   *Effects of climate change on epidemiology*

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

192   range expansion is not straightforward, either for apparently emerging parasites such as the

193   rumen fluke *Calicophoron daubneyi* [23] or for other helminths, and this undermines

194   attempts to predict future challenges to farming. Given the multiple interacting factors that

195   drive parasite epidemiology, research should embed parasitic disease in wider studies of

196   climate change mitigation and adaptation in livestock and mixed agricultural systems [24].

197

198   *Improved diagnostics for epidemiological monitoring*

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

200

201   Various methods have been extensively documented to recover infective stages of GINs and

202   flukes from herbage or tracer animals, followed by microscopic counting and identification

203   by morphological or molecular methods [25]. However, modern quantitative and qualitative

204   molecular methods have not been sufficiently adapted for rapid estimation of the level of

205   parasite challenge. Success would have clear applications to parasite management as well as

206   improving the feasibility of field studies to test epidemiological and evolutionary predictions.

207

208   **Section II: Economic and environmental impacts**

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

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

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

212   ruminant?

213   15. How can environmental impacts of anthelmintics be properly measured, including on

214   non-target fauna, and ecosystem functioning and service provision?

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

216

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

242 sustainability, and the economic benefits of helminth control in less monetised farming

243 systems, remain challenging [33].

244

245 **Section III: Effects on host behaviour and welfare**

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

247 18. How does parasitism affect animal behaviour?

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

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

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

251 22. Are animals better off and healthier with some worms, rather than none? Studies are

252 biased towards negative effects on hosts, and neglect potentially positive outcomes at

253 individual and population levels.

254

255 *Measuring behavioural impacts of parasitism*

256 Research into the impacts of helminth infections on the behaviour and welfare of livestock

257 has largely focused on aspects of direct economic importance in ruminant livestock [34], and

258 is lagging behind research into the behavioural and welfare impacts of parasites in other host-

259 parasite systems [35]. The impact of subclinical helminth infection on host behaviour and

260 welfare indicators remains largely understudied, perhaps in part because such subclinical

261 effects can be hard to detect and difficult to separate from those of other disorders. Still,

262 changes can be more objectively measured today using new technologies. Thus, advances in

263 electronic technology (e.g. 3D accelerometers), offers novel tools to monitor and detect host

264 welfare and behavioural responses to parasitism and to link these to targeted control efforts

265 [36]. Further, positive behaviours that allow livestock to avoid or suppress infection, such as

266 self-medication and selective grazing, may be identified as markers to selectively breed for

267 'behavioural' resistance [37]. The importance of behaviour as a defence strategy against GIN

268 is recognized in goats [38], but empirical evidence for selectively breeding grazing animals to

269 develop this trait is so far lacking.

270

271 *Helminth infection is not necessarily negative*

272 Studies to date focus on negative effects on hosts, and neglect potentially positive outcomes

273 of helminth infections, such as regulatory roles at scales ranging from gut microbiomes and

274 inflammation [39] to entire grazing systems [40]. Studies taking a more holistic view of the

275 consequences of infection for individual and group health would be timely given changes in

276 farming systems and increasing societal concern in many countries for the welfare and

277 environmental costs of modern farming practices.

278

279 **Section IV: Host–helminth-microbiome interactions**

280 23. How do gastrointestinal parasites communicate in the gut?

281 24. How does interaction between different helminths in co-infection affect the immune

282 system of the host and the development of disease?

283 25. Are there associations between animals' microbiomes and helminth communities, and do

284 they matter?

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

286 versa?

287 27. To what extent do co-infections between helminths and other specific pathogens, e.g.

288 liver fluke and bovine tuberculosis; gastrointestinal nematodes and paratuberculosis;

289 lungworms and respiratory pathogens; influence health outcomes for livestock and human

290 health?

291

Helminths interact with other infections but consequences vary

293 The ability of helminths to influence the host response and dictate disease outcomes of co-

294 infections is an active area of research within parasitology [41], in which many questions

295 remain unanswered. In classical co-infection scenarios, a co-evolutionary dynamic between

296 the vertebrate host, helminths and microbiome is thought to result from complex adaptations

297 of each of the three components [42]. Research into helminth-microbiota co-infections in

298 livestock hosts is in its early stages, raising questions about whether a host's microbiome and

299 helminth community interact and communicate, how any such interaction impacts on the host

300 immune response to both natural infections and vaccines, and whether it can be manipulated

301 to enhance host immunity. Inconsistencies exist between different studies, methodologies and

302 approaches, but a growing body of evidence from humans and rodent model systems has

303 identified helminth-associated changes in gut microbiota [43,44]. It remains to be established

304 whether this occurs as a direct effect of the parasite itself or as a secondary effect driven by

305 the host and its immune response, or perhaps both [44]. Clearly a better understanding of co-

306 infections (in consideration also of different helminths, or of helminths and micro-

307 organisms), the mechanisms they invoke, and, importantly, their impact on the health and

308 productivity of livestock is required [45,46]. A systems biology approach, drawing insights

309 from diverse host environments (e.g. including livestock and wildlife systems), pathogen

310 combinations and stages of infection [41,44,47-49] offers promise to advance our knowledge

311 and identify potential alternative strategies for parasite control. A truly holistic view would

312 also include the impact that helminths and their control may have on other diseases and their

313 detection, including zoonoses [50].

314

315 **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*

14

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

366   dimensions, including characteristics of the main parasites of concern now and in future,

367   production aims and farm management system, and should guard against unintended

368   consequences for co-infections.

369

370   **Section VI: Development and detection of anthelmintic resistance**

371   38. What is the relative importance of management *versus* environmental factors in

372   determining the development of anthelmintic resistance in livestock?

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

374   resistance?

375   40. What changes in genes other than those encoding for the immediate drug target, such as

376   transporters and drug metabolism, are involved in anthelmintic resistance?

377   41. What do we understand about the fitness costs of anthelmintic resistance and how can

378   they be measured?

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

380   43. Is there a link between the size of the *refugia* needed to slow or prevent anthelmintic

381   resistance and the molecule and formulation used (e.g. persistent *versus* non-persistent)?

382   44. Can combination anthelmintic formulations be designed that are more effective and that

383   limit resistance development?

384   45. Do differences in life history traits and reproductive strategy affect the risk for

385   development of anthelmintic resistance?

386   46. What is the effect of long-lasting drug formulations such as moxidectin injections or

387   benzimidazole boluses on the development of anthelmintic resistance in sheep, goats and

388   cattle?

389   47. Is treatment of ectoparasites with macrocyclic lactone drugs an important driver of

390   anthelmintic resistance in sheep and goats?

391 48. Are *in-vitro*/genetic/laboratory methods for detection of anthelmintic resistance desirable,

392 reachable and applicable for all anthelmintic drug groups?

393 49. How can we best improve monitoring of the efficacy of current control methods (e.g.

394 through diagnostics, resistance testing and surveillance)?

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

396 51. What is the true status of anthelmintic resistance in less-studied livestock systems, e.g.

397 ascarids in pigs and poultry?

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

399 circumstances?

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

401 minimises bias?

402

403 *Mechanisms and processes in resistance*

404 The evolution of AR in parasitic helminths is considered to be driven by a range of parasite

405 intrinsic and extrinsic factors [57]. To the former belong drug- and species-specific

406 susceptibility, effective parasite population size and genetic variability. External factors

407 include treatment frequency and intensity, and the size of the *refugia*, which strongly depend

408 on local management and environmental determinants. How these factors interact and

409 influence the development of a phenotypically resistant worm population is currently largely

410 unclear. Also the molecular mechanisms of AR are not well established for most

411 combinations of helminth species and drug groups. Nevertheless, in the case of the

412 benzimidazoles, a well-developed understanding of the resistance mechanism has enabled

413 molecular tools to be established for AR detection, which can be used to elucidate patterns of

414 spread of resistance on a broad scale for ruminants [58]. The situation in pigs and poultry,

415 however, is barely known [59].

416

Towards better diagnosis of anthelmintic resistance

There is a great need to extend our knowledge on the driving forces of AR development, to

establish field applicable and meaningful resistance detection tools, and hence to provide

more up-to-date and reliable information on the occurrence of AR. In an era of revolution of

technology in the diagnostic industries, improvement of the "old-fashioned" faecal egg count

reduction test (FECRT), for example through use of pooled faecal samples [60-62], or

eventually automation, has great potential to allow more rapid, labour-efficient and remote

assessment of AR. This remains a worthwhile aim because definitive molecular tests remain

elusive for most drug groups and helminth species. Better tests would enable AR to be

distinguished from other causes of poor efficacy, including through the administration of sub-

standard generic compounds [63]. Links between AR in livestock and humans, through

zoonotic transmission of resistant parasites such as *Ascaris* spp., and in terms of potential for

shared understanding of mechanisms and approaches to limit AR, remain underexplored.

Section VII: Practical management of anthelmintic resistance

When to intervene against resistance

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

55. When should drug combinations be used to combat anthelmintic resistance, and when

not?

Optimal usage of anthelmintic drugs in the face of AR should be tailor-made and consider

parasite species, host species, farm management and climatic factors [2,3]. Deciding how to

extend the lifetime of drugs, either before or after some resistance is evident [64,65], requires

consideration of actual levels of AR and how fast AR spreads given selection pressures

441   imposed by factors such as drug type and number of treatments, whether treatments are

442   targeted or not, and the presence of *refugia* [66,67].

443

444   *Refugia in principle and practice*

445   56. What empirical evidence is there that *refugia* slow down the development of drug

446   resistance?

447   57. What proportion of a helminth population must be left in *refugia* in order to slow the

448   development of anthelmintic resistance?

449   58. How does the level of *refugia* influence the detection and spread of resistant phenotypes

450   in different hosts, different parasites and different treatment systems?

451   59. Is there a role for *refugia* in control of liver fluke?

452   60. If *refugia* are not appropriate for all parasite species that display drug resistance, what

453   realistic alternatives exist for those situations?

454   61. Can anthelmintic resistance be practically reversed, e.g. through targeted selective

455   treatment, good grazing management, or reseeding (community replacement or dilution)

456   approaches?

457

458   The concept of *refugia* is widely accepted, but is still surrounded by several assumptions and

459   approximations, and the level of *refugia* required may depend on prevailing (e.g. climatic)

460   circumstances [68]. *Refugia* as a concept has been mainly applied to GIN but its role in

461   resistance management in other helminths needs further research. Also, the extent to which

462   *refugia* might play a role in the reversal of AR [65], as opposed to just slowing its

463   development [69] is currently far from clear, as is the practical usefulness of community

464   replacement strategies for re-gaining anthelmintic susceptibility on farms [70].

465

What to do about known resistance status?

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

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].

541

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.

591   The interactions between different PSM and between PSM and anthelmintics remain largely

592   unexplored and contrasting results have been described [85]. The development of refined

593   methods to assess the anthelmintic potential of plant compounds are needed. Some

594   practicalities around use of PSM on farms also need to be addressed, such as regulation of

595   mode of distribution, level of inclusion in feed, and potential residues in animal products.

596

597   *Other alternative control methods*

598   89. What are the main obstacles (not only technical) to the development of new technologies

599   to control helminths of livestock?

600   90. Can we target helminth stages outside the host to achieve control, e.g. killing stages

601   on pasture or manipulating intermediate host biology?

602   91. Are there basic processes in egg hatching or larval development that can be manipulated

603   to aid control?

604

605   The objective of integrated parasite management is to limit the level of parasitism below

606   acceptable limits while delaying the emergence of drug resistance. This aim has motivated

607   the search for and refined use of PSM as well as other alternatives to commercial chemical

608   anthelmintics, including vaccines, host resistance and grazing management [86]. Good

609   pasture management is one of the major means to limit the intake of infective larvae by

610   animals, e.g. by use of parasite-free fields, pasture rotations, and alternation of grazing

611   animals, taking into account the seasonal dynamics of helminth transmission. Manipulation

612   of environmental conditions that play a role in the development of intermediate stages may

613   also be a form of alternative control. For example, grazing away from wet pasture, where

614   feasible, markedly lowers the risk of *F. hepatica* infection, due to lower exposure to infection

615   near intermediate snail host habitats [87]. Free-living stages of GIN may also be targeted

616   directly, for instance through application of urea or other nitrogen-based fertilisers to pasture

617   [88,89]. Certain bioactive forages, e.g. chicory, are also thought to hamper the development

618   of free-living stages, either by reducing the fitness of eggs excreted from hosts grazing on the

619   forage, or because the physico-chemical properties of the forage reduce larval availability on

620   herbage [90]. Biological control based on nematode trapping fungi (*Duddingtonia flagrans*,

621   *Arthrobotrys musiformis*) or entomopathogenic bacteria can also reduce the number of free

622   living stages on pasture and the level of host infections; results from mechanical stressors

623   such as a diatomaceous earth are less promising [91,92]. Refined understanding of the

624   mechanisms of action of these non-chemotherapeutic alternative control methods and how

625   they might be applied to manage helminth populations on farms provide potentially fruitful

626   avenues for further research.

627

628   **Section X: Stakeholder engagement**

629   *New decision support tools for helminth control*

630   92. How can different novel control methods for helminths be integrated effectively and in a

631   way that is simple enough for farmers to implement?

632   93. Can helminth control decision support tools be integrated effectively in farm or pasture

633   management software?

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

635   poor?

636   95. Is research in veterinary helminth infections reaching livestock farmers in developing

637   countries and, if so, what is the impact?

638

639   Veterinary parasitologists working with livestock might consider extending their efforts from

640   task-oriented research targeting the development and refinement of helminth control

641   strategies, and advance towards advice-oriented health management practices. To achieve this

642   would involve answering some key research questions around development of decision

643   support tools that can integrate different worm control strategies into whole-farm

644   management [9], taking into account also the regulatory frameworks and economic

645   environments in which farmers operate. Researchers are now looking further down this road

646   and questioning how their strategies will fit best into the whole farm environment and how

647   decision tools can be integrated, for example in farm management practices and decision

648   support systems. Even though there is considerable knowledge on available complementary

649   strategies, substantial deficits remain around knowledge exchange and transfer, and the

650   research community is becoming increasingly aware that better promotion of such strategies

651   to the farmers is crucial for their success [93].

652

653   *Understanding farmer behaviour to support effective knowledge exchange*

654   96. What factors drive anthelmintic treatment decisions by farmers?

655   97. How can the importance of a strategic approach to helminth treatment be more effectively

656   promoted among producers, especially when drug resistance is not yet an issue?

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

658   farmers?

659   99. How does the attitude of farmers with respect to accepting and implementing parasite

660   control measures differ between countries and cultures?

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

662   use?

663

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

665   insights into factors that drive farmers' decisions about worm control and use those insights

666   to develop communication strategies to promote sustainable worm control practices [94].

667   Major reasons why suggested solutions often do not fit with farmers' views are that they are

668   highly complex (involving language and cultural barriers) and not cost-efficient (too

669   expensive), encompass conflicting interests (e.g. intensive versus extensive farming systems)

670   and priorities, and may require contradictory management interventions at farm level.

671   Consequently, educating and motivating farmers and adopting a multi-actor approach are key

672   issues. Stronger empirical evidence for the effectiveness of integrated parasite control

673   strategies and their compatibility with performance targets is key to adoption [94,95].

674   Researchers must understand the fundamental and instrumental relationships between

675   individual farmers' values, behaviour and perception of risk, to stimulate and qualify the

676   farmer's decision-making in a way that will increase the farmer's satisfaction and subjective

677   well-being, and not only narrow metrics around performance or financial return [26,96].

678   Factors that influence farmers' behaviour are not limited to technical or practical issues such

679   as ease of use or price, but also include less 'tangible' factors such as the opinion of others or

680   habits [97-99]. Barriers and incentives for sustainable worm control that were identified in

681   such quantitative and qualitative studies may vary between farmer types (e.g. sheep farmers

682   vs. dairy cattle farmers) or between countries. Moreover, before these factors can be

683   translated into communication strategies, they should first be validated in communication

684   experiments [100]. In the literature on changing animal health behaviour, the majority

685   comprises studies that investigate the factors that influence behaviour intention, which at best

686   suggests which social intervention could be developed to change this intended behaviour, but

687   rarely assess whether such intervention could work [101]. Finally, human behaviour (and thus

688   also farmer behaviour) is also strongly influenced by unconscious processes, such as

689   intuition, which has not yet been studied in the context of sustainable parasite control [102].

690   As a community, veterinary parasitologists need to adopt a trans-disciplinary approach,

691   together with epidemiologists, social scientists, economists and others (including livestock

692   scientists, grassland management experts, conservationists, processors, retailers and farmers

693   themselves), which will result in a better understanding of farmer behaviour and motivation

694   with respect to drug treatments and parasite control.

695

696   **Concluding remarks**

697   The questions listed above were the result of an attempt to elicit research priorities from a

698   wider constituency than in more usual review formats, which are typically led by a small

699   number of established experts. It was anticipated that this would yield a wider-ranging set of

700   potential research topics and directions, less constrained by forces that shape disciplinary

701   academic consensus. In the event, the topics and questions are broadly similar to those raised

702   in recent expert reviews [1,4,6-8,103], and reflect a high level of current concern over the

703   biology of AR, how to measure and manage it, and the quest for alternative options for the

704   control of helminths on farms. This is perhaps not surprising given that improved helminth

705   management is a key goal of most researchers in the discipline, whether they lean toward

706   fundamental or applied research, and that AR is the main threat to existing control strategies.

707   Control of helminth infections in mainstream farming systems with fewer chemical inputs is

708   a topical challenge and one that will require new research, technologies, and perhaps

709   economic goals [1].

710   Questions around helminth epidemiology, management of AR, and alternative control

711   approaches including *refugia*, were frequently repeated in the original list (see supplemental

712   material), for example being posed more than once for different parasite or host taxa. To

713   achieve feasible smaller research projects as envisaged at the start of this exercise, many of

714   the questions could be broken back down again to specific taxa, both to produce system-

715 specific knowledge and applied solutions, and to explore the generality of conclusions from

716 more studied contexts. Challenges in tropical or less developed countries yielded few specific

717 questions, as did those related to pig and poultry production. Participation was strongly

718 skewed towards European countries, in spite of efforts to be inclusive, possibly as a result of

719 the European roots of LiHRA, under whose auspices the exercise was conducted (Box 1).

720 Nevertheless, questions submitted from outside Europe focused on similar areas, and almost

721 all of the final questions are relevant across wide geographic areas and often globally. The

722 voting round (Box 2) might also have distorted results and led to the loss of original but less

723 popular ideas from the final list, though such a step was necessary to limit numbers of

724 questions and exclude some to which answers are already well-known. The full list is

725 included as supplemental material to this article.

726 While not definitive, the final list of 100 questions serves to indicate current concerns among

727 the livestock helminth research community, and highlights several areas in which existing

728 understanding is poor while fresh advances now appear possible. The questions might serve

729 to encourage or inspire work in those areas. For example, early career researchers might

730 peruse the list to identify topics on which short or starter projects might have

731 disproportionately high impact on the state of knowledge. It would be instructive to repeat

732 this exercise in future, to determine how many of the questions have been answered, and

733 whether the state of knowledge, the enabling technologies, or the problems of the day have

734 moved sufficiently to generate different gaps and priorities. In the meantime, as a community,

735 there is clearly work to be done to explore interesting questions whose answers are highly

736 relevant to the ability of humankind to feed itself in the future while respecting the global

737 environment and the health and welfare of the animals that sustain us.

738

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754

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1002   Deciding where public and private research spending will have the greatest impact is a

1003   complex process involving multiple interests. Often, *ad hoc* expert groups are created to

1004   provide decision makers with advice over specific topics. In addition, over the last decade

1005   several initiatives have emerged at European and global levels to foster international

1006   discussions and apply a structured approach to the identification of research gaps and

1007   priorities in the animal health domain, including livestock helminthology in Europe.

1008   DISCONTOOLS (www.discontools.eu) is a publicly funded, open-access database to assist

1009   public and private funders of animal health research and researchers in identifying research

1010   gaps and planning future research [104]. The database contains research gaps as well as a gap

1011   scoring and prioritization model for more than 50 infectious diseases of animals. The

1012   information is provided by disease-specific expert groups and updated on a 5-year cycle.

1013   The DISCONTOOLS database acts as a key resource for the STAR-IDAZ International

1014   Research Consortium on animal health (www.star-idaz.net), comprising research funders and

1015   programme owners from Europe, Asia, Australasia, the Americas, Africa and the Middle

1016   East, as well as international organisations, and includes representation from veterinary

1017   pharmaceutical companies. Members coordinate their research programmes to address agreed

1018   research needs, share results, and together seek new and improved animal health strategies

1019   for at least 30 priority diseases, infections or issues. These include candidate vaccines,

1020   diagnostics, therapeutics and other animal health products, procedures and key scientific

1021   information and tools to support risk analysis and disease control. STAR-IDAZ develops

1022   road maps on how to achieve these new animal health strategies.

1023   The Animal Task Force (ATF) (www.animaltaskforce.eu) is a European public-private

1024   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.

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

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

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

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

1092 engagement from some parts of the world (e.g. Australia, South America) in spite of efforts

1093 to reach those regions, and a European bias in the core group and arguably therefore in the

1094 outcome, with a strong focus on anthelmintic resistance. The bias to Europe might be

1095 symptomatic of greater relevant research activity here than on other continents, but whatever

1096 the reason risks perpetuating focus on existing areas of strength in exactly the way this

1097 exercise sought to oppose. We exhort researchers in low and middle income countries in

1098 particular to seize the initiative in driving forward the research agenda to meet the needs in

1099 their countries, using researchers established elsewhere to support their efforts but not

1100 necessarily to determine the questions addressed or approaches used. It is also recommended

1101 that future elicitation exercises with similar aims make creative attempts to engage those who

1102 are less disposed to contribute, and further lessen the role of authors, for example by reducing

1103 the size and participation of the core group.

1104

Glossary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1154   **Star-IDAZ** – International Research Consortium on animal health (www.star-idaz.net),
1155   comprising research funders and programme owners from Europe, Asia, Australasia, the
1156   Americas, Africa and the Middle East, as well as international organisations, and including
1157   representation from veterinary pharmaceutical companies. Members coordinate their research
1158   programmes to address agreed research needs, share results, and together seek new and
1159   improved animal health strategies for at least 30 priority diseases, infections or issues. See
1160   Box 1.
1161   **Targeted selective treatment (TST)** – the treatment of only some individual animals within
1162   a group at one time, instead of the more common whole-group treatment, where all animals
1163   in the group are treated simultaneously.
1164   **Targeted treatment (TT)** – treatment of animals at a time selected to either minimise the
1165   impact on the selection for anthelmintic resistance, or to maximise animal productivity.
1166   **Zoonoses** – infections that can be transferred from animals to humans.
1167

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 parasitical 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?

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

93. What regulates egg production in females and can we suppress female egg production sufficiently to provide an epidemiological advantage?

94. Will breeding for resistance (low FECs and high production potential) drive nematode adaptation towards increased fecundity to compensate?

Helminth biology and epidemiology - diagnostics

95. How can I see or detect that my flock or herd is infected by helminths?

96. How can we improve the diagnosis of *Fasciola* spp?

97. How far are we away from tests in the live animal for immature fluke and Nematodirus infestations?

98. How to predict a clinical case of dictyocaulosis in cattle?

99. In a flock or herd, which sampling protocol should be followed for the diagnosis of helminth infections?

100. Is a mixed species of GINs in one animal difficult to control compared to an infected animal with one GIN species?

101. Is there some general European strategy for (manual) of examination of livestock for helminthoses, before a treatment? Which methods are used in particular countries?

102. What new technologies are used to detect infections by helminths in livestock?

103. When will automated diagnostic tools/technologies be really available for on-farm diagnosis?

104. Which user-friendly parameters can help the farmer (or veterinarian) to make informed decisions on helminth control in young stock?

105. Why are faecal egg counts not at all times a good parameter to assess worm counts of strongyles?

Economic and environmental impacts

106. From an economical and ecological point of view, what helminths do farmers think are the most important? How would they list them?

107. How accurately can we predict changes in the seasonality and magnitude of risk?

108. How can helminth control be integrated in farm management in a cost-efficient way?

109. How can we better assess production and health impacts of helminths?

110. How can you measure environmental impacts of anthelmintics?

111. Can we put an economic dollar value on the importance of a more strategic approach to GIN treatment to producers?

112. How does helminth control impact on the environment (MLs on microorganisms, environmental schemes etc)?

113. How important is it for us to chase subclinical GI nematodes in grazing beef cattle with low FEC?

114. How the three main farming systems (capitalistic, entrepreneur-type, peasant / small farming / family farming) modify through values and technicity the parasite community?

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

116. Is there a market space to promote livestock products raised without (or with limited) use of anthelmintics?

117. Is there an association between countries or regions that have high levels of *Fasciola* and level of income in those countries / regions?

118. Is there an impact in the environment by the overuse of anthelmintics over the past decades?

1367 119. Should we be advising anthelmintic treatment of dairy cows with antibodies to *O.*
1368 *ostertagi* but no clinical signs? Is a potential 1kg/d increase in yield worth the cost, time and
1369 increased use of anthelmintics?
1370 120. What are the consequences on productions of helminth infections (including pigs and
1371 poultry)?
1372 121. What are the costs (financial, human and welfare) of anthelmintic resistance?
1373 122. What are the economics of GIN and Fasciola infection in cattle?
1374 123. What are the long-term impacts of anthelmintics on beneficial dung fauna and their
1375 functioning?
1376 124. What is the economic burden of helminths of livestock in each country around the
1377 world, in 2017?
1378 125. What is the economic impact of anthelmintic resistance in livestock?
1379 126. What is the economical impact of strongyle infections in ruminants?
1380 127. What is the real impact of parasitic gastroenteritis on small ruminant production?
1381 128. What is the true financial cost of helminth infection?
1382 129. What is the true on farm economic impact of sheep (and cattle?) bred for resistance and
1383 is it a viable option for future breeding? E.g. impact on reducing pasture contamination /
1384 subsequent parasite challenge?
1385 130. Which factors determine the role of helminth infections in the whole-farm economic
1386 context?
1387 131. Will the benefits of helminth control of livestock for global environmental sustainability
1388 become as important as economic benefits are now when promoting our research?
1389 132. Does the control of helminths reduce the net methane emission over the lifetime of a
1390 ruminant?
1391
1392 **Effects on host behaviour and welfare**
1393 133. Are animals better off and healthier with some worms, rather than none?
1394 134. Can we select for host behaviour to control helminth infections?
1395 135. Do ruminant parasites change the behaviour of the host?
1396 136. Do ruminants graze complex vegetation selectively to avoid nematode infection?
1397 137. Do ruminants self-medicate by selectively grazing plants with anthelmintic compounds?
1398
1399 138. How can parasites be beneficial to hosts (individually or in terms of population or
1400 species levels)? All studies are biased on the negative effect on host.
1401 139. How can we develop animal production supportive and welfare based control strategies
1402 in soil-transmitted helminth infections?
1403 140. How does parasitism affect animal behaviour and can we use changes in behaviour as a
1404 way of identifying those that need treatment?
1405 141. How can we measure the impact of helminth infections on livestock welfare?
1406
1407 **Host-helminth-microbiome interactions**
1408 142. Are there associations between animals' microbiomes and helminth communities?
1409 143. Can the alteration of gut microbiota influence the immunity to parasites in livestock?
1410 144. How does the gut microbiome interact with GI helminths and does it matter?
1411 145. How important are other microorganisms and multispecies interactions for driving
1412 parasitic disease in livestock?
1413 146. How is the pathobiome considered in the host genetic selection scheme?
1414 147. How strong is the influence of microbiota on nematode diversity?
1415 148. What is the importance of climate change, helminth infections and immune response to
1416 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
1428 **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
1465 **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 moxidection injections of 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

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 macrocylic
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 reseeding
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
1758 **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?