

Helminths and microbes within the vertebrate gut – not all studies are created equal

Journal:	<i>Parasitology</i>
Manuscript ID	PAR-2019-0112.R2
Manuscript Type:	Review
Date Submitted by the Author:	16-Jun-2019
Complete List of Authors:	Cortes, Alba; Cambridge Veterinary School, Department of Veterinary Medicine Peachey, Laura; Cambridge Veterinary School, Department of Veterinary Medicine Jenkins, Timothy; Cambridge Veterinary School, Department of Veterinary Medicine Scotti, Riccardo; Cambridge Veterinary School, Department of Veterinary Medicine Cantacessi, Cinzia; Cambridge Veterinary School, Department of Veterinary Medicine; University of Cambridge
Key Words:	Helminth-microbiota interactions, Microbial richness, Microbial diversity, Human studies, Gastrointestinal parasites

SCHOLARONE™
Manuscripts

Invited review

1
2
3 **Helminths and microbes within the vertebrate gut –**
4 **not all studies are created equal**

5
6 Alba Cortés¹, Laura E. Peachey^{1,2}, Timothy P. Jenkins¹, Riccardo Scotti¹ and Cinzia Cantacessi¹

7
8 ¹*Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES,*
9 *Cambridge, United Kingdom*

10 ²*Bristol Veterinary School, Faculty of Health Sciences, University of Bristol, Langford House,*
11 *Langford, BS40 5DU, Bristol, United Kingdom*

12
13
14 **Running title:** Helminth-microbiota interactions in the human gut

15
16
17 **Corresponding author:**

18 Cinzia Cantacessi

19 *Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES,*
20 *Cambridge, United Kingdom*

21 Tel. +44 (0) 1223 760541

22 Fax. +44 (0)1223 337610

23 E-mail: cc779@cam.ac.uk

24
25 *4,648 words*

29 SUMMARY

30 The multifaceted interactions occurring between gastrointestinal (GI) parasitic helminths and the
31 host gut microbiota are emerging as a key area of study within the broader research domain of
32 host-pathogen relationships. Over the past few years, a wealth of investigations has demonstrated
33 that GI helminths interact with the host gut flora, and that such interactions result in modifications
34 of the host immune and metabolic statuses. Nevertheless, whilst selected changes in gut microbial
35 composition are consistently observed in response to GI helminth infections across several host-
36 parasite systems, research in this area to date is largely characterised by inconsistent findings.
37 These discrepancies are particularly evident when data from studies of GI helminth-microbiota
38 interactions conducted in humans from parasite-endemic regions are compared. In this review,
39 we provide an overview of the main sources of variance that affect investigations on human-
40 helminth-gut microbiota interactions and propose a series of methodological approaches that,
41 whilst accounting for the inevitable constraints of human fieldwork, are aimed at minimising
42 confounding factors and draw biologically meaningful interpretations from highly variable
43 datasets.

44

45 1. INTRODUCTION

46 A plethora of experimental evidence supports a key role of infections by gastrointestinal (GI)
47 helminth parasites in shaping the composition of the vertebrate gut microbiota, with significant
48 implications for local and systemic host immunity (reviewed by Brosschot and Reynolds, 2018).
49 For instance, recent studies have partly attributed the parasite-associated qualitative and/or
50 quantitative alterations to host GI microbial profiles to the ability of GI helminths to stimulate the
51 initial onset of T-regulatory (Treg) immune responses (cf. Cantacessi *et al.* 2014; Reynolds *et al.*
52 2014; Giacomini *et al.* 2015, 2016; Zaiss *et al.* 2016). On the other hand, other studies have
53 reported associations between acute helminth infections and gut microbiota imbalances (=
54 dysbiosis) characterised by significant expansion of populations of putative pro-inflammatory
55 bacteria (e.g. Rausch *et al.* 2013; Jenkins *et al.* 2018a; Schneeberger *et al.* 2018a); these
56 observations have lent credit to the hypothesis that helminth-associated alterations of gut
57 microbiota composition may lead to both localised and systemic consequences for the host
58 organism, that include immunopathology and exacerbated malnutrition in at-risk subjects from
59 parasite-endemic areas (reviewed by Glendinning *et al.* 2014; Houlden *et al.* 2015; Cattadori *et*
60 *al.* 2016).

61 Over the past decade, newly acquired knowledge of the impact that GI helminth infections exert
62 on the vertebrate gut microbial composition and metabolism has contributed to a better
63 understanding of parasite systems biology and host-pathogen interactions (reviewed by Peachey
64 *et al.* 2017; Leung *et al.* 2018; Rapin and Harris *et al.* 2018), and has been proposed as a first step
65 towards the identification and development of novel strategies of parasite control based on the
66 targeted manipulation of the host gut microbiota (cf. Peachey *et al.* 2017). Nevertheless, for
67 humans in particular, progress in this field of research is greatly impaired by the impact of several
68 confounding factors that inevitably affect studies conducted in naturally infected individuals
69 (Mutapi, 2015; Chabé *et al.* 2017). In this review, we summarise current knowledge of GI
70 helminth-microbiome interactions in humans under natural conditions of infection, identify
71 similarities and differences between datasets and provide an overview of the confounding factors
72 that may affect the interpretation of findings.

73 2. HUMAN-HELMINTH-GUT MICROBIOTA INTERACTIONS IN REAL-WORLD
74 SCENARIOS

75 In endemic areas for helminthiases, the vast majority of infected individuals harbour multiple
76 helminth species, often occupying different niches of the host organism (Hotez *et al.* 2010).
77 Whilst polyparasitism is often regarded as a major confounding factor in investigations of
78 parasite-microbiota interactions conducted in humans under natural conditions of infection
79 (Cooper *et al.* 2013; Jenkins *et al.* 2017; Martin *et al.* 2018; Rosa *et al.* 2018), findings from these
80 studies are key to assessing the impact that GI helminths exert on gut microbiota homeostasis in
81 a ‘real-world’ scenario. Nevertheless, several factors should be considered when interpreting
82 results obtained from individuals infected by multiple helminth species. First, anthropometric
83 (e.g. age and gender) and anthropologic variables (e.g. ethnicity, diet and occupation) are well
84 known to profoundly impact the ‘baseline’ composition of the human gut microbiota (Sekirov *et al.*
85 *al.* 2010; Yatsunenکو *et al.* 2012) (cf. Fig. 1); therefore, the enrolment of large cohorts of
86 individuals is often necessary in order to achieve sufficient statistical power and avoid
87 uninformative and/or misleading results (Kelly *et al.* 2015). However, in many studies, the
88 number of individuals enrolled and samples analysed is inevitably dictated by logistical and
89 financial constraints. In these instances, population-related variables that impact gut microbiota
90 composition may contribute substantially to inconsistencies among findings from different studies
91 (cf. Fig. 1). For instance, a negative association between colonisation by the whipworm *Trichuris*
92 *trichiura* and the abundance of bacteria belonging to the genus *Prevotella* in the faeces of infected
93 individuals has been reported in two separate studies conducted in Malaysia (Lee *et al.* 2014;
94 Ranaman *et al.* 2016), while other studies conducted in Ecuador, and Liberia and Indonesia,
95 respectively, have failed to identify significant variations in faecal populations of *Prevotella* in
96 individuals either solely infected by *T. trichiura* or co-infected with other species of soil-
97 transmitted helminths (STHs) (Cooper *et al.* 2013; Martin *et al.* 2015; Rosa *et al.* 2018).

98 In addition, whilst Rosa and co-authors (2018) detected several distinctive features in the gut
99 microbial profiles of helminth-harboring individuals that were specifically associated to single

100 infections with the hookworm *Necator americanus*, the roundworm *Ascaris lumbricoides* or *T.*
101 *trichiura*, such features were inconsistent between two independent cohorts of helminth-infected
102 volunteers from Liberia and Indonesia; this discrepancy suggests that other yet undetermined
103 environmental factors may contribute to qualitative and quantitative alterations of the gut
104 microbial profiles of helminth-infected individuals from different geographical areas. In contrast,
105 an association between the abundance of selected bacterial taxa and infections by one or more
106 STHs could be consistently detected in samples from both Liberian and Indonesian cohorts (Rosa
107 *et al.* 2018). These taxa included bacteria belonging to the genera *Olsenella* and *Allobaculum*,
108 which were expanded in the gut microbiota of helminth-infected individuals when compared to
109 that of uninfected controls. To the best of our knowledge, the study by Rosa *et al.* (2018) was the
110 first to report a link between infections by STHs and the abundance of these bacterial genera in
111 the human gut. Interestingly, in mice suffering from metabolic syndrome, administration of
112 probiotics was followed by expansion of populations of *Olsenella* and/or *Allobaculum*, and a
113 reduction in systemic and/or local gut inflammatory responses (Wang *et al.* 2015). Moreover,
114 *Allobaculum* spp. are putative producers of anti-inflammatory short-chain fatty acids (Greetham
115 *et al.* 2004), and are severely reduced in the gut of mice genetically predisposed to spontaneous
116 colitis (Pérez-Muñoz *et al.* 2014). This knowledge led Rosa *et al.* (2018) to hypothesize that these
117 bacteria may play a yet undetermined role in the anti-inflammatory properties of parasitic
118 helminths, and reinforce the proposition that the interactions between hosts, parasites and gut
119 microbiota are multidirectional and should be approached in a holistic manner (e.g. Cortés *et al.*
120 2018; Leung *et al.* 2018). Interestingly, in contrast to evidence acquired in human hosts, a negative
121 association between the genus *Allobaculum* and colonisation by GI helminths has been observed
122 in a mouse model of chronic trichuriasis (Holm *et al.* 2015), in which Th1-mediated immune
123 responses are dominant (reviewed by Cliffe and Grencis, 2004), as well as in mice with patent
124 infection by the blood fluke *Schistosoma mansoni* (Jenkins *et al.* 2018a), in which migrating eggs
125 are responsible for the onset of marked Th2-mediated inflammatory responses (reviewed by
126 Pearce and MacDonald, 2002). The immune-molecular mechanisms *via* which members of the
127 genus *Allobaculum* may regulate local and systemic inflammation are still unclear (Greetham *et*

128 *al.* 2004; Pérez-Muñoz *et al.* 2014; Wang *et al.* 2015). Nonetheless, current data showing
129 reductions in populations of *Allobaculum* alongside helminth-associated gut inflammation
130 supports the hypothesis raised by Rosa *et al.* (2018); in the future, rodent models of GI helminth
131 infections whose gut microbiota is deprived of, and subsequently recolonised with, the genus
132 *Allobaculum* could be exploited to investigate the potential involvement of these bacteria in
133 parasite-mediated immunomodulation.

134 Beside the intrinsic variability of the human gut microbiota, studies conducted under natural
135 conditions of helminth colonisation are likely to be affected by factors linked to the different
136 combinations of infecting species and their relative abundances. For instance, in a study
137 conducted in a cohort of Ecuadorian children, the specific features detected in the gut microbial
138 profiles of subjects co-infected with *T. trichiura* and *A. lumbricoides* could not be identified in
139 the microbiota of *Trichuris*-only infected individuals (Cooper *et al.* 2013). Similarly, selected
140 microbial features that were observed in studies conducted in human volunteers with mono-
141 specific infections with, for instance, *A. lumbricoides*, could not be detected in the gut microbiota
142 of subjects harbouring the same parasite alongside other helminth species (e.g. *T. trichiura* and
143 *N. americanus*) (Rosa *et al.* 2018), thus suggesting that a complex interplay exists between the
144 host gut and its macro- and microbiota, that might be difficult to replicate in experimental settings.
145 Furthermore, current evidence obtained from animal models of helminth infections indicates that
146 worm burdens can impact the nature and/or the magnitude of parasite-associated alterations in gut
147 microbial composition (Wu *et al.* 2012; Peachey *et al.* 2018). Nevertheless, such evidence is not
148 yet available for human infections, in which parasite burdens may range from low to very high in
149 endemic areas (Barbour and Kafetzaki, 1991; Churcher *et al.* 2005).

150 Another frequent constraint of investigations conducted in cohorts of human subjects with natural
151 helminth infections is the limited availability of ‘genuine’ negative controls, i.e. individuals from
152 the same communities of parasite-infected subjects who lack previous exposure to infections by
153 parasitic helminths. Instead, individuals with no evidence of patent helminth infections are
154 inevitably enrolled as control subjects (e.g. Cooper *et al.* 2013; Lee *et al.* 2014; Jenkins *et al.*

155 2017; Rosa *et al.* 2018); nevertheless, studies in helminth-infected individuals subjected to
156 anthelmintic treatment, as well as in primates and pigs exposed to *Trichuris* spp., have shown that
157 parasite-associated alterations in gut microbial communities can persist, at least partly, in absence
158 of active infections (Broadhurst *et al.* 2012; Wu *et al.* 2012; Cooper *et al.* 2013; Kay *et al.* 2015;
159 Schneeberger *et al.* 2018a). These data call for caution when interpreting differences between the
160 gut microbial profiles of helminth-infected and uninfected volunteers from the same communities.
161 In addition, patent infections are often diagnosed using stool-based microscopic methods, that are
162 known for their relatively low sensitivity and that may yield false negative results, e.g. in case of
163 intermittent shedding of eggs and/or larvae (O'Connell and Nutman, 2016). Recently, Rosa *et al.*
164 (2018) used quantitative real-time PCR to diagnose STH infections in individuals subjected to gut
165 microbiota profiling, indicating that this technique may represent a robust and sensitive
166 alternative to microscopic methods, since it provides users with the ability to semi-quantify
167 burdens of different helminth species from minute amounts of DNA template. However, in spite
168 of their higher sensitivity, molecular methods rely on the use of primers that selectively target the
169 parasite species of interest, thus impairing the simultaneous detection of potential (asymptomatic
170 or subclinical) co-infections with other helminth and/or non-helminth pathogens (O'Connell and
171 Nutman, 2016). Indeed, the impact of protozoa on the gut microbial diversity and composition
172 has been clearly demonstrated in humans and other vertebrates (reviewed by Chabé *et al.* 2017;
173 Stensvold and van der Giezen, 2018). Furthermore, a recent study conducted in a cohort of
174 Colombian schoolchildren reported common features in the faecal microbial composition of
175 subjects co-infected with helminths and protozoans and mono-parasitized with the flagellate
176 *Giardia intestinalis* compared to uninfected individuals (Toro-Londono *et al.* 2019). Whilst the
177 mechanisms *via* which each group of parasites alters the host gut flora, as well as the nature of
178 such alterations, are yet to be determined, these findings support the need to conduct additional
179 diagnostic tests on stool samples from helminth-infected cohorts, as well as the corresponding
180 uninfected subjects, in order to rule out the influence of concomitant bacterial, viral and/or
181 protozoan infections that may be responsible for the changing gut microbial profiles of these
182 individuals (cf. Chabé *et al.* 2017).

183 Nevertheless, in spite of the several confounding factors outlined above (cf. Fig. 1), observational
184 studies in helminth endemic areas have proven useful for the identification of significant
185 associations between parasite colonisation and the gut microbial profiles of humans under natural
186 conditions of infection. Importantly, studies conducted in these communities provide excellent
187 opportunities to evaluate the effect(s) that parasite removal (e.g. via the administration of broad-
188 spectrum anthelmintics) exert(s) on the gut microbiota of previously infected individuals, thus
189 contributing cues to understand the causality of helminth-microbiota relationships.

190 3. IMPACT OF DEWORMING ON THE HUMAN GUT MICROBIOTA

191 The implementation of mass drug administration programmes in endemic areas for STHs and
192 schistosomiasis offers opportunities to elucidate potential mechanisms *via* which parasitic
193 helminths modulate the host gut microbiota. For instance, qualitative and quantitative changes in
194 gut microbial profiles that are caused by direct interactions between parasites and gut bacteria
195 may be expected to rapidly reverse following parasite removal, whilst long-lasting alterations are
196 likely to result from indirect interplay mediated by the host immune system (Houlden *et al.* 2015;
197 Su *et al.* 2018). Nevertheless, such investigations are also generally constrained by the presence
198 of several confounding factors that include not only the host- and parasite-dependent variables
199 outlined above, but also variations linked to the use of different drugs and treatment regimes
200 (Schneeberger *et al.* 2018b), as well as time of sampling post-anthelmintic treatment (Houlden *et*
201 *al.* 2015) (Fig. 1). The latter in particular may profoundly affect findings from these studies, as
202 the presence of tissue lesions caused by e.g. parasite feeding activity and location (e.g. blood-
203 feeders *vs.* non blood-feeders and luminal *vs.* tissue dwellers) are likely to influence the timespan
204 between helminth removal and microbiome recovery (reviewed by Leung *et al.* 2018). Moreover,
205 for ethical reasons, data from these experiments is often biased by the lack of placebo-treated
206 control groups. These limitations may be at least partially responsible for the differences between
207 findings from studies aimed at elucidating the effect of deworming on the gut microbiota of
208 helminth-infected volunteers; notwithstanding, it is worth noting that, in instances where

209 deworming-associated changes in human gut microbial profiles were detected, these were
210 generally moderate (Ramanan *et al.* 2016; Martin *et al.* 2018; Schneeberger *et al.* 2018b).

211 Consistent with this, a recent study conducted on faecal samples collected from a rural community
212 in Indonesia reported that the composition of the gut microbiota of individuals repeatedly treated
213 with either albendazole or placebo (for 21 months) resembled that of samples collected from the
214 same subjects prior to treatment, rather than that of uninfected controls (Rosa *et al.* 2018).
215 Moreover, a parallel investigation conducted on the same cohort of individuals detected reduced
216 populations of *Prevotella* in albendazole-treated subjects in which complete deworming did not
217 occur, compared to placebo-treated individuals with patent helminth infections (Martin *et al.*
218 2018). Intriguingly, failure of albendazole treatment was accompanied by a dominance of *T.*
219 *trichiura* (over other helminth species) in these subjects, while placebo-treated individuals
220 maintained a diverse macrobiota (i.e. multiple helminth infections); hence, differences in the
221 composition of the GI macrobiota (i.e. species present and their relative abundances) between
222 albendazole- and placebo-treated individuals could account for variations in the composition of
223 the intestinal microflora of these subjects (Martin *et al.* 2018). Significant associations between
224 colonisation by *T. trichiura* and *Prevotella* abundance were not observed in the Indonesian cohort
225 (Martin *et al.* 2018; Rosa *et al.* 2018). However, negative associations between whipworm
226 infections and *Prevotella* abundance had been detected previously in two independent studies
227 conducted in Malaysia (Lee *et al.* 2014; Ramanan *et al.* 2016). In particular, Ramanan and co-
228 authors (2016) observed that, following albendazole treatment, expansion of *Prevotella*
229 populations in the human faecal microbiota was related to reduced *T. trichiura* faecal egg counts.

230 In contrast, no significant associations between helminth infection and abundance of bacteria
231 belonging to the genus *Prevotella* was reported in a study investigating the impact of parasite
232 colonisation and successful treatment with a combination of albendazole and ivermectin on the
233 faecal microbial profiles of a cohort of *Trichuris*-infected children from Ecuador (Cooper *et al.*
234 2013), nor in a group of helminth-infected adults from Sri Lanka treated with pyrantel pamoate
235 (Jenkins *et al.* 2017). Similarly, no qualitative or quantitative changes to faecal microbial

236 composition were observed in two cohorts of schoolchildren from Côte d'Ivoire and Zimbabwe
237 infected by *S. mansoni* and *S. haematobium*, respectively, following treatment with praziquantel
238 (Kay *et al.* 2014; Schneeberger *et al.* 2018a). However, successful elimination of *S. mansoni* was
239 associated with a higher abundance of *Fusobacterium* spp. pre-treatment, as well as 24 hrs post-
240 treatment (Schneeberger *et al.* 2018a).

241 Whilst drug administration in endemic regions may result in effective elimination of helminth
242 infections, potential co-infecting protozoan parasites are not susceptible to anthelmintic
243 treatment; this, together with the sub-standard hygienic and sanitary conditions that generally
244 characterise these areas and that result in continuous re-exposure to infective helminth
245 developmental stages (Campbell *et al.* 2018), impairs the full assessment of the consequences of
246 helminth removal on the composition of the human gut microbiota. To the best of our knowledge,
247 thus far, a single study has investigated the effects of chronic infections by a GI helminth,
248 *Strongyloides stercoralis*, and anthelmintic treatment on the composition of the faecal microbiota
249 and metabolome of humans from a non-endemic area of Europe, where parasite transmission had
250 been interrupted (Jenkins *et al.* 2018b). Treatment with ivermectin resulted in compositional
251 changes of the faecal microbiota (analysed 6 months post-treatment), which partially resembled
252 that of uninfected control subjects (Jenkins *et al.* 2018b); in particular, alpha diversity [= a
253 measure of the number of bacterial species present in a given microbial community (richness) and
254 their relative abundance (evenness)] was reduced in the microbiota of individuals post-treatment
255 (although statistical significance was not achieved) and accompanied by expanded populations of
256 potentially pathogenic bacteria (Jenkins *et al.* 2018b). In addition, the faecal metabolic profiles
257 obtained from samples collected post-ivermectin treatment shared features with both those
258 obtained from samples collected pre-treatment and from uninfected controls (Jenkins *et al.*
259 2018b); this observation led Jenkins *et al.* (2018b) to hypothesise that, following parasite removal
260 and over time, both gut microbiota and metabolome may revert to the original pre-infection state.
261 Multiple factors, including but not limited to those outlined above, may contribute to the
262 discrepancies observed between the findings from this work and those that reported no or minor

263 effects of anthelmintic treatment on the gut microbiome of helminth-infected humans (Cooper *et*
264 *al.* 2013; Ramanan *et al.* 2016; Martin *et al.* 2018; Rosa *et al.* 2018; Schneeberger *et al.* 2018a,b).

265 Despite the limitations outlined above, studies of GI helminth-microbiota relationships conducted
266 in endemic areas for helminthiasis have provided repeated evidence of the perturbations that
267 parasites and anthelmintic treatment exert on the equilibrium of resident populations of gut
268 bacteria and on gut homeostasis. However, the identification of common signatures across studies
269 remains key to designing future experiments, e.g. in animal models of helminth infections, that
270 may assist the elucidation of the mechanisms that underpin the interactions between GI helminths,
271 the gut microbiota and the host immune system.

272 4. DO COMMON SIGNATURES EXIST ACROSS STUDIES OF HOST-HELMINTH- 273 MICROBIOTA INTERACTIONS?

274 The identification of gut microbial signatures that occur reproducibly across several host-GI
275 helminth systems is crucial for designing novel anti-helminth intervention strategies based on the
276 manipulation of the gut microbiota (Peachey *et al.* 2017). Studies conducted in animal models of
277 helminth infections are expected to assist the identification of such signatures, as well as the direct
278 (i.e. parasite-mediated) and/or indirect (i.e. immune-mediated) mechanisms that govern helminth-
279 microbiota interactions (Cortés *et al.* 2018); nevertheless, the inconsistencies that characterise
280 studies of helminth-microbiota relationships published to date make such a task highly
281 challenging. Indeed, for patterns to be identified, fluctuations in selected populations of gut
282 microbes must be interpreted in light of the physical and immunological alterations of the mucosal
283 environment in which such alterations occur (Leung *et al.* 2018). For instance, expanded
284 populations of *Lactobacillaceae* have been repeatedly detected following infection with several
285 species of parasitic helminths in several host species (Reynolds *et al.* 2014; Duarte *et al.* 2015;
286 Holm *et al.* 2015; Houlden *et al.* 2015; Cattadori *et al.* 2016; Jenkins *et al.* 2018a; Kim *et al.*
287 2018), and could thus be considered as a ‘consistent alteration’ in gut microbiota composition
288 upon helminth colonisation. However, key differences exist between host-parasite pairs
289 investigated in the studies that have reported such an outcome. Indeed, whilst populations of

290 *Lactobacillaceae* promote regulatory responses in mice infected by *Heligmosomoides polygyrus*
291 *bakeri* (Reynolds et al. 2014), a lack of correlation between *Lactobacillaceae* abundance and Treg
292 populations has been observed in other host-parasites systems, such as mice chronically infected
293 with *T. muris* and rabbits infected with *Trichostrongylus retortaeformis*, in which the expansion
294 of populations of gut *Lactobacillaceae* upon helminth infection occurs in an environment
295 dominated by Th1-mediated immune responses (Holm et al. 2015; Houlden et al. 2015; Cattadori
296 et al. 2016). These differences suggest that alternative mechanisms may regulate the
297 differentiation and development of adaptive immune responses in each host-parasite system
298 (Houlden et al. 2015), and thus that similar alterations in gut microbiota composition may result
299 in different consequences that are dependent on the microenvironment where these changes occur.
300 Notwithstanding, the interactions between hosts, helminths and the gut microbiota are likely
301 multifaceted and multidirectional, and therefore the potential consequences that selected
302 compositional changes in gut microbiota exert on host homeostasis are only one aspect of these
303 complex interplay. For instance, a common yet undetermined mechanism may determine the
304 expansion of *Lactobacillaceae* in the gut of helminth-infected hosts.

305 On the other hand, apparent ‘contradictory’ findings across studies may result from fundamental
306 differences between gut compartments under investigation. For instance, *Prevotella* spp. was
307 expanded in the abomasum and faeces of sheep infected by abomasal trichostrongyles (i.e.
308 *Haemonchus contortus* and *Teladorsagia circumcincta*; Li et al. 2016; Cortés et al. in
309 preparation), whilst the same taxa were reduced in the faeces of a range of host species, including
310 mice, humans and horses, infected by nematodes residing in the large intestine, i.e. *Trichuris* spp.
311 and cyathostomins, respectively (Lee et al. 2014; Houlden et al. 2015; Peachey et al. submitted).
312 It must be noted, however, that whilst increased abomasal pH favours *Prevotella* overgrowth in
313 the abomasum (De Nardi et al. 2016; Li et al. 2016), the same taxa are likely to be exposed to a
314 dramatically different microenvironment in the large intestine that may determine the contraction
315 of these bacterial groups. In addition, given the functional dissimilarities between the abomasal
316 and colonic microbiota, such alterations are expected to result in fundamentally different

317 outcomes for the homeostasis of each of these gut compartments (Ley *et al.* 2008), and hence
318 comparisons are, in our opinion, unwarranted.

319 In parallel to species of bacteria with functions that may vary depending on the gut compartment,
320 multiple taxa share the same functions in different microenvironments (Lozupone *et al.* 2012);
321 therefore, it is plausible that, even though inconsistencies are detected across studies, these may
322 result in similar functional alterations in the host-parasite pairs being compared. For instance,
323 recent studies in mouse and humans infected with *S. mansoni* have reported the expansion of
324 different genera of bacteria with pro-inflammatory functions in the gut microbiota of the
325 respective hosts (Jenkins *et al.* 2018a; Schneeberger *et al.* 2018a). These observations lend credit
326 to the hypothesis that the functional role of the gut microbiota in helminth infections could be far
327 less 'diverse' than the taxonomic associations reported thus far. For this hypothesis to be
328 confirmed or confuted, a better understanding of the function(s) of each bacterial taxon inhabiting
329 the different gut compartments in a range of host species is needed. To this aim, the integration
330 of metagenomic, metabolomic and metatranscriptomic technologies, alongside traditional
331 microbiology and microscopy techniques, may assist to achieve a holistic picture of the impact of
332 GI helminth infections on the functions of the human gut microbiota, and its significance for
333 disease pathophysiology and overall host health (Wang *et al.* 2015).

334 5. CURRENT NEEDS AND FUTURE DIRECTIONS

335 Understanding the complex interactions between GI helminths and their vertebrate hosts is pivotal
336 for advancing our knowledge of the fundamental biology of these parasites and the diseases they
337 cause (see Peachey *et al.* 2017; Leung *et al.* 2018; Rapin and Harris *et al.* 2018 for reviews).
338 Whilst the role of the gut microbiota in host-parasite relationships has long been overlooked,
339 current knowledge of the key roles that resident bacteria play in host health and disease, together
340 with recent technical advancements for microbiota profiling, have boosted research in this area.
341 This is currently leading to increasing evidence of a role for the gut microbiota in the immune
342 regulatory properties of helminth parasites (Cantacessi *et al.* 2014; Reynolds *et al.* 2014;
343 Giacomini *et al.* 2015, 2016; Zaiss *et al.* 2016). In addition, data collected to date points towards

344 a likely role of the gut microflora in the immunopathology of selected GI helminth infections that
345 awaits experimental validation. Trying to untangle the relevance of particular fluctuations of
346 specific bacterial taxa on infection outcome is challenging; nevertheless, currently available data
347 suggest that low-intensity, long-term helminth infections are commonly linked to high microbial
348 diversity and predominance of bacteria typically associated with gut health. Conversely, high-
349 intensity, acute infections are often associated to gut dysbiosis, characterised by reduced alpha
350 diversity and an increase in pro-inflammatory and often opportunistic pathogens (Peachey *et al.*
351 2017). However, for this knowledge to be exploited in translational studies, further investigations
352 in both natural and experimental settings are needed to distinguish spurious results from genuine
353 helminth-microbiota associations (Peachey *et al.* 2017), and mechanistic studies in animal models
354 of helminth infections are necessary to dissect the causality of these relationships (cf. Cortés *et*
355 *al.* 2018). Importantly, minimising variations between studies is crucial to warrant meaningful
356 comparisons between datasets.

357 Whilst reducing the variability amongst samples collected from naturally helminth-infected
358 humans may be difficult to achieve, the enormous impact that differences in technical and
359 experimental approaches (from sample collection to bioinformatics and biostatistical analyses)
360 exert on the overall variation detected across studies can be reduced (Figs. 1 and 2; Lindgreen *et*
361 *al.* 2017; Costea *et al.* 2017; Golob *et al.* 2017). In particular, a range of bioinformatics pipelines
362 are available for the analysis of high-throughput amplicon and metagenomics sequence datasets
363 that include, e.g., different sequence-processing tools and reference databases for sequence
364 annotation that could yield slightly different results (Lindgreen *et al.* 2017; Golob *et al.* 2017).
365 For instance, the use of validated open microbiome analysis packages such Multiplexed Analysis
366 of Projections by Sequencing (MAPseq) (Matias Rodrigues *et al.* 2017) or QIIME2
367 (<https://qiime2.org/>) may assist accurate taxonomic classifications of bacterial 16S rRNA
368 amplicon datasets; similarly, sequence annotation should rely on the use of regularly updated
369 reference databases. Amongst these, SILVA (<https://www.arb-silva.de/>) (Quast *et al.* 2013)
370 enables sensitive annotations of bacterial rRNA sequence data (Almeida *et al.* 2018). The use of

371 such standardized analysis workflows and reference databases for sequence annotation might
372 prove extremely useful to increase consistency across studies and enable researchers to identify
373 common and/or unique features between the gut microbiota of different host-parasite systems
374 which, in turn, might assist to better understand the mechanisms that regulate helminth-microbiota
375 relationships.

376 The consequences that elucidating such mechanisms may exert on future strategies of parasite
377 control are two-fold. First, disentangling the potential contribution of the gut flora to the
378 pathogenesis of the infection is necessary in order to discover and develop new strategies to
379 contrast helminth-associated pathology. Second, understanding the microbiota-dependent
380 mechanisms by which parasitic helminths are able to modulate host immune responses and
381 suppress inflammation may assist the discovery of novel immune-regulatory therapeutics against
382 chronic inflammatory disorders of the GI tract that may act in synergy with helminth-based
383 therapy (see Peachey *et al.* 2017 and Rapin and Harris, 2018 for reviews). However, in order for
384 this new knowledge to be fully exploited in translational research, further studies that thoroughly
385 consider inclusion/exclusion criteria for the selection of participants, include appropriate controls,
386 and follow standardised experimental and data analysis protocols are necessary, and will allow to
387 disentangle the potential influence of parasite-, drug- and/or population-dependent variables in
388 each setting (Fig. 2).

389

390

391 ACKNOWLEDGEMENTS

392 The authors would like to thank Professor R. Stephen Phillips for helpful suggestions on the draft
393 manuscript.

394 FINANCIAL SUPPORT

395 AC is supported by a postdoctoral fellowship from Fundación Alfonso Martín Escudero (Madrid,
396 Spain). LEP is supported by funding from the Horserace Betting Levy Board (HBLB) and TPJ
397 by scholarships by the Biotechnology and Biological Sciences Research Council (BBSRC) of the
398 United Kingdom. Research in the CC laboratory is supported by grants by the Royal Society and
399 the Isaac Newton Trust.

400

For Peer Review

401 REFERENCES

- 402 **Almeida A, Mitchell AL, Tarkowska A and Finn RD** (2018) Benchmarking taxonomic
403 assignments based on 16S rRNA gene profiling of the microbiota from commonly sampled
404 environments. *GigaScience* **7**, 1-10.
- 405 **Barbour AD and Kafetzaki M** (1991) Modeling the overdispersion of parasite loads.
406 *Mathematical Biosciences* **107**, 249-253.
- 407 **Broadhurst MJ, Ardeshir A, Kanwar B, Mirpuri J, Gundra UM, Leung JM, Wiens KE,**
408 **Vujkovic-Cvijin I, Kim CC, Yarovinsky F, Lerche NW, McCune JM and Loke P** (2012)
409 Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the
410 inflammatory signature and mucosal microbiota of the colon. *PLoS Pathogens* **8**, e1003000.
- 411 **Brosschot TP and Reynolds LA** (2018) The impact of a helminth-modified microbiome on host
412 immunity. *Mucosal Immunology* **11**, 1039-1046.
- 413 **Campbell SJ, Biritwum NK, Woods G, Velleman Y, Fleming F and Stothard JR** (2018)
414 Tailoring water, sanitation, and hygiene (WASH) targets for soil-transmitted helminthiasis and
415 schistosomiasis control. *Trends in Parasitology* **34**, 53-63.
- 416 **Cantacessi C, Giacomini P, Croese J, Zakrzewski M, Sotillo J, McCann L, Nolan MJ,**
417 **Mitreva M, Krause L and Loukas A** (2014) Impact of experimental hookworm infection on the
418 human gut microbiota. *The Journal of Infectious Diseases* **210**, 1431-1434.
- 419 **Cattadori IM, Sebastian A, Hao H, Katani R, Albert I, Eilertson KE, Kapur V, Pathak A**
420 **and Mitchell S** (2016) Impact of helminth infections and nutritional constraints on the small
421 intestine microbiota. *PLoS One* **11**, e0159770.
- 422 **Chabé M, Lokmer A and Segurel L** (2017) Gut protozoa: friends or foes of the human gut
423 microbiota? *Trends in Parasitology* **33**, 925-934.
- 424 **Churcher TS, Ferguson NM and Basáñez MG** (2005) Density dependence and
425 overdispersion in the transmission of helminth parasites. *Parasitology* **131**(Pt 1), 121-132.

- 426 **Cliffe LJ and Grecis RK** (2004) The *Trichuris muris* system: a paradigm of resistance and
427 susceptibility to intestinal nematode infection. *Advances in Parasitology* **57**, 255-307.
- 428 **Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M and Parkhill J** (2013) Patent
429 human infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in
430 the faecal microbiota. *PLoS One* **8**, e76573.
- 431 **Cortés A, Toledo R and Cantacessi C** (2018) Classic models for new perspectives: delving into
432 helminth-microbiota-immune system interactions. *Trends in Parasitology* **34**, 640-654.
- 433 **Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen**
434 **M, Hercog R, Jung FE, Kultima JR, Hayward MR, Coelho LP, Allen-Vercoe E, Bertrand**
435 **L, Blaut M, Brown JRM, Carton T, Cools-Portier S, Daigneault M, Derrien M, Druesne A,**
436 **de Vos WM, Finlay BB, Flint HJ, Guarner F, Hattori M, Heilig H, Luna RA, van Hylckama**
437 **Vlieg J, Junick J, Klymiuk I, Langella P, Le Chatelier E, Mai V, Manichanh C, Martin JC,**
438 **Mery C, Morita H, O'Toole PW, Orvain C, Patil KR, Penders J, Persson S, Pons N, Popova**
439 **M, Salonen A, Saulnier D, Scott KP, Singh B, Slezak K, Veiga P, Versalovic J, Zhao L,**
440 **Zoetendal EG, Ehrlich SD, Dore J and Bork P** (2017) Towards standards for human fecal
441 sample processing in metagenomic studies. *Nature Biotechnology* **35**, 1069-1076.
- 442 **De Nardi R, Marchesini G, Li S, Khafipour E, Plaizier KJ, Ganesella M, Ricci R,**
443 **Andrighetto I and Segato S** (2016) Metagenomic analysis of rumen microbial population in
444 dairy heifers fed a high grain diet supplemented with dicarboxylic acids or polyphenols. *BMC*
445 *Veterinary Research* **12**, 29-016-0653-4.
- 446 **Duarte AM, Jenkins TP, Latrofa MS, Giannelli A, Papadopoulos E, de Carvalho LM, Nolan**
447 **MJ, Otranto D and Cantacessi C** (2016) Helminth infections and gut microbiota - a feline
448 perspective. *Parasites & Vectors* **9**, 625-016-1908-4.
- 449 **Giacomin P, Zakrzewski M, Croese J, Su X, Sotillo J, McCann L, Navarro S, Mitreva M,**
450 **Krause L, Loukas A and Cantacessi C** (2015) Experimental hookworm infection and escalating

- 451 gluten challenges are associated with increased microbial richness in celiac subjects. *Scientific*
452 *Reports* **5**, 13797.
- 453 **Giacomin P, Zakrzewski M, Jenkins TP, Su X, Al-Hallaf R, Croese J, de Vries S, Grant A,**
454 **Mitreva M, Loukas A, Krause L and Cantacessi C** (2016) Changes in duodenal tissue-
455 associated microbiota following hookworm infection and consecutive gluten challenges in
456 humans with coeliac disease. *Scientific Reports* **6**, 36797.
- 457 **Glendinning L, Nausch N, Free A, Taylor DW and Mutapi F** (2014) The microbiota and
458 helminths: sharing the same niche in the human host. *Parasitology* **141**, 1255-1271.
- 459 **Golob JL, Margolis E, Hoffman NG and Fredricks DN** (2017) Evaluating the accuracy of
460 amplicon-based microbiome computational pipelines on simulated human gut microbial
461 communities. *BMC Bioinformatics* **18**, 283-017-1690-0.
- 462 **Greetham HL, Gibson GR, Giffard C, Hippe H, Merkhoffer B, Steiner U, Falsen E and**
463 **Collins MD** (2004) *Allobaculum stercoricanis* gen. nov., sp. nov., isolated from canine
464 feces. *Anaerobe* **10**, 301-307.
- 465 **Holm JB, Sorobetea D, Kiilerich P, Ramayo-Caldas Y, Estelle J, Ma T, Madsen L,**
466 **Kristiansen K and Svensson-Frej M** (2015) Chronic *Trichuris muris* infection decreases
467 diversity of the intestinal microbiota and concomitantly increases the abundance of
468 Lactobacilli. *PLoS One* **10**, e0125495.
- 469 **Hotez PJ, Alvarado M, Basanez MG, Bolliger I, Bourne R, Boussinesq M, Brooker SJ,**
470 **Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fevre EM, Furst T, Halasa YA,**
471 **Jasrasaria R, Johns NE, Keiser J, King CH, Lozano R, Murdoch ME, O'Hanlon S, Pion SD,**
472 **Pullan RL, Ramaiah KD, Roberts T, Shepard DS, Smith JL, Stolk WA, Undurraga EA,**
473 **Utzinger J, Wang M, Murray CJ and Naghavi M** (2014) The global burden of disease study
474 2010: interpretation and implications for the neglected tropical diseases. *PLoS Neglected Tropical*
475 *Diseases* **8**, e2865.

- 476 **Houlden A, Hayes KS, Bancroft AJ, Worthington JJ, Wang P, Grencis RK and Roberts**
477 **IS** (2015) Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host
478 microbiota and metabolome: effects reversed by pathogen clearance. *PLoS One* **10**, e0125945.
- 479 **Jenkins TP, Formenti F, Castro C, Piubelli C, Perandin F, Buonfrate D, Otranto D, Griffin**
480 **JL, Krause L, Bisoffi Z and Cantacessi C** (2018b) A comprehensive analysis of the faecal
481 microbiome and metabolome of *Strongyloides stercoralis* infected volunteers from a non-
482 endemic area. *Scientific Reports* **8**, 15651.
- 483 **Jenkins TP, Peachey LE, Ajami NJ, MacDonald AS, Hsieh MH, Brindley PJ, Cantacessi C**
484 **and Rinaldi G** (2018a) *Schistosoma mansoni* infection is associated with quantitative and
485 qualitative modifications of the mammalian intestinal microbiota. *Scientific Reports* **8**, 12072.
- 486 **Jenkins TP, Rathnayaka Y, Perera PK, Peachey LE, Nolan MJ, Krause L, Rajakaruna RS**
487 **and Cantacessi C** (2017) Infections by human gastrointestinal helminths are associated with
488 changes in faecal microbiota diversity and composition. *PLoS One* **12**, e0184719.
- 489 **Kay GL, Millard A, Sergeant MJ, Midzi N, Gwisai R, Mduluzi T, Ivens A, Nausch N,**
490 **Mutapi F and Pallen M** (2015) Differences in the faecal microbiome in *Schistosoma*
491 *haematobium* infected children vs. uninfected children. *PLoS Neglected Tropical Diseases* **9**,
492 e0003861.
- 493 **Kelly BJ, Gross R, Bittinger K, Sherrill-Mix S, Lewis JD, Collman RG, Bushman FD and**
494 **Li H** (2015) Power and sample-size estimation for microbiome studies using pairwise distances
495 and PERMANOVA. *Bioinformatics* **31**, 2461-2468.
- 496 **Kim JY, Kim EM, Yi MH, Lee J, Lee S, Hwang Y, Yong D, Sohn WM and Yong TS** (2018)
497 Intestinal fluke *Metagonimus yokogawai* infection increases probiotic *Lactobacillus* in mouse
498 cecum. *Experimental Parasitology* **193**, 45-50.
- 499 **Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, Gundra UM, Cho I, Bonneau R,**
500 **Blaser MJ, Chua KH and Loke P** (2014) Helminth colonization is associated with increased
501 diversity of the gut microbiota. *PLoS Neglected Tropical Diseases* **8**, e2880.

- 502 **Leung JM, Graham AL and Knowles SCL** (2018) Parasite-microbiota interactions with the
503 vertebrate gut: synthesis through an ecological lens. *Frontiers in Microbiology* **9**, 843.
- 504 **Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML,**
505 **Tucker TA, Schrenzel MD, Knight R and Gordon JI** (2008) Evolution of mammals and their
506 gut microbes. *Science* **320**, 1647-1651.
- 507 **Li RW, Li W, Sun J, Yu P, Baldwin RL and Urban JF** (2016) The effect of helminth infection
508 on the microbial composition and structure of the caprine abomasal microbiome. *Scientific*
509 *Reports* **6**, 20606.
- 510 **Lindgreen S, Adair KL and Gardner PP** (2016) An evaluation of the accuracy and speed of
511 metagenome analysis tools. *Scientific Reports* **6**, 19233.
- 512 **Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK and Knight R** (2012) Diversity,
513 stability and resilience of the human gut microbiota. *Nature* **489**, 220-230.
- 514 **Martin I, Djuardi Y, Sartono E, Rosa BA, Supali T, Mitreva M, Houwing-Duistermaat JJ**
515 **and Yazdanbakhsh M** (2018) Dynamic changes in human-gut microbiome in relation to a
516 placebo-controlled anthelmintic trial in Indonesia. *PLoS Neglected Tropical Diseases* **12**,
517 e0006620.
- 518 **Mutapi F** (2015) The gut microbiome in the helminth infected host. *Trends in Parasitology* **31**,
519 405-406.
- 520 **Matias Rodrigues JF, Schmidt TSB, Tackmann J and von Mering C** (2017) MAPseq: highly
521 efficient k-mer search with confidence estimates, for rRNA sequence analysis. *Bioinformatics* **33**,
522 3808-3810.
- 523 **O'Connell EM and Nutman TB** (2016) Molecular diagnostics for soil-transmitted
524 helminths. *The American Journal of Tropical Medicine and Hygiene* **95**, 508-513.
- 525 **Peachey LE, Jenkins TP and Cantacessi C** (2017) This gut ain't big enough for both of us. Or
526 is it? Helminth-microbiota interactions in veterinary species. *Trends in Parasitology* **33**, 619-632.

- 527 **Peachey LE, Molena RA, Jenkins TP, Di Cesare A, Traversa D, Hodgkinson JE and**
528 **Cantacessi C** (2018) The relationships between faecal egg counts and gut microbial composition
529 in UK thoroughbreds infected by cyathostomins. *International Journal for Parasitology* **48**, 403-
530 412.
- 531 **Pearce EJ and MacDonald AS** (2002). The immunobiology of schistosomiasis. *Nature Reviews*
532 *Immunology* **2**, 499-511.
- 533 **Pérez-Muñoz ME, Bergstrom K, Peng V, Schmaltz R, Jiménez-Cardona R, Marsteller N,**
534 **McGee S, Clavel T, Ley R, Fu J, Xia L and Peterson DA** (2014) Discordance between changes
535 in the gut microbiota and pathogenicity in a mouse model of spontaneous colitis. *Gut Microbes* **5**,
536 286-295.
- 537 **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J and Glöckner FO**
538 (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-
539 based tools. *Nucleic Acids Research* **41**, 590-596.
- 540 **Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, Honda K, Gause WC, Blaser**
541 **MJ, Bonneau RA, Lim YA, Loke P and Cadwell K** (2016) Helminth infection promotes
542 colonization resistance via type 2 immunity. *Science* **352**, 608-612.
- 543 **Rapin A and Harris NL** (2018) Helminth-bacterial interactions: cause and consequence. *Trends*
544 *in Immunology* **39**, 724-733.
- 545 **Rausch S, Held J, Fischer A, Heimesaat MM, Kühl AA, Bereswill S and Hartmann S** (2013)
546 Small intestinal nematode infection of mice is associated with increased enterobacterial loads
547 alongside the intestinal tract. *PLoS One* **8**, e74026.
- 548 **Reynolds LA, Smith KA, Filbey KJ, Harcus Y, Hewitson JP, Redpath SA, Valdez Y, Yebra**
549 **MJ, Finlay BB and Maizels RM** (2014) Commensal-pathogen interactions in the intestinal tract:
550 Lactobacilli promote infection with, and are promoted by, helminth parasites. *Gut Microbes* **5**,
551 522-532.
- 552 **Rosa BA, Supali T, Gankpala L, Djuardi Y, Sartono E, Zhou Y, Fischer K, Martin J, Tyagi**
553 **R, Bolay FK, Fischer PU, Yazdanbakhsh M and Mitreva M** (2018) Differential human gut
554 microbiome assemblages during soil-transmitted helminth infections in Indonesia and
555 Liberia. *Microbiome* **6**, 33-018-0416-5.
- 556 **Schneeberger PHH, Coulibaly JT, Gueuning M, Moser W, Coburn B, Frey JE and Keiser**
557 **J** (2018b) Off-target effects of tribendimidine, tribendimidine plus ivermectin, tribendimidine
558 plus oxantel-pamoate, and albendazole plus oxantel-pamoate on the human gut
559 microbiota. *International Journal for Parasitology Drugs and Drug Resistance* **8**, 372-378.

- 560 **Schneeberger PHH, Coulibaly JT, Panic G, Daubenberger C, Gueuning M, Frey JE and**
561 **Keiser J** (2018a) Investigations on the interplays between *Schistosoma mansoni*, praziquantel
562 and the gut microbiome. *Parasites & Vectors* **11**, 168-018-2739-2.
- 563 **Sekirov I, Russell SL, Antunes LC and Finlay BB** (2010) Gut microbiota in health and
564 disease. *Physiological Reviews* **90**, 859-904.
- 565 **Stensvold CR and van der Giezen M** (2018) Associations between gut microbiota and common
566 luminal intestinal parasites. *Trends in Parasitology* **34**, 369-377.
- 567 **Su C, Su L, Li Y, Long SR, Chang J, Zhang W, Walker WA, Xavier RJ, Cherayil BJ and**
568 **Shi HN** (2018) Helminth-induced alterations of the gut microbiota exacerbate bacterial
569 colitis. *Mucosal Immunology* **11**, 144-157.
- 570 **Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van-Hyleckama Vlieg JE, Strissel**
571 **K, Zhao L, Obin M and Shen J** (2015) Modulation of gut microbiota during probiotic-mediated
572 attenuation of metabolic syndrome in high fat diet-fed mice. *The ISME Journal* **9**, 1-15.
- 573 **Wang WL, Xu SY, Ren ZG, Tao L, Jiang JW and Zheng SS** (2015) Application of
574 metagenomics in the human gut microbiome. *World Journal of Gastroenterology* **21**, 803-814.
- 575 **World Health Organisation** (2006) Preventive chemotherapy in human helminthiasis:
576 coordinated use of anthelmintic drugs in control interventions: a manual for health professionals
577 and programme managers. Geneva: WHO; 2006.
- 578 **Wu S, Li RW, Li W, Beshah E, Dawson HD and Urban JF Jr.** (2012) Worm burden-dependent
579 disruption of the porcine colon microbiota by *Trichuris suis* infection. *PLoS One* **7**, e35470.
- 580 **Yatsunencko T, Rey FE, Manary MJ, Trehan I, Domínguez-Bello MG, Contreras M, Magris**
581 **M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J,**
582 **Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R and Gordon**
583 **JI** (2012) Human gut microbiome viewed across age and geography. *Nature* **486**, 222-227.
- 584
- 585

586 **FIGURE LEGENDS**

587 Fig. 1 Sources of variation and confounding factors potentially impacting the outcome of studies
588 of human-helminth-gut microbiota interactions in helminth-endemic regions.

589 Fig. 2 Proposed approaches aimed at reducing the methodological sources of variation
590 surrounding investigations of human-helminth-gut microbiota interactions.

For Peer Review

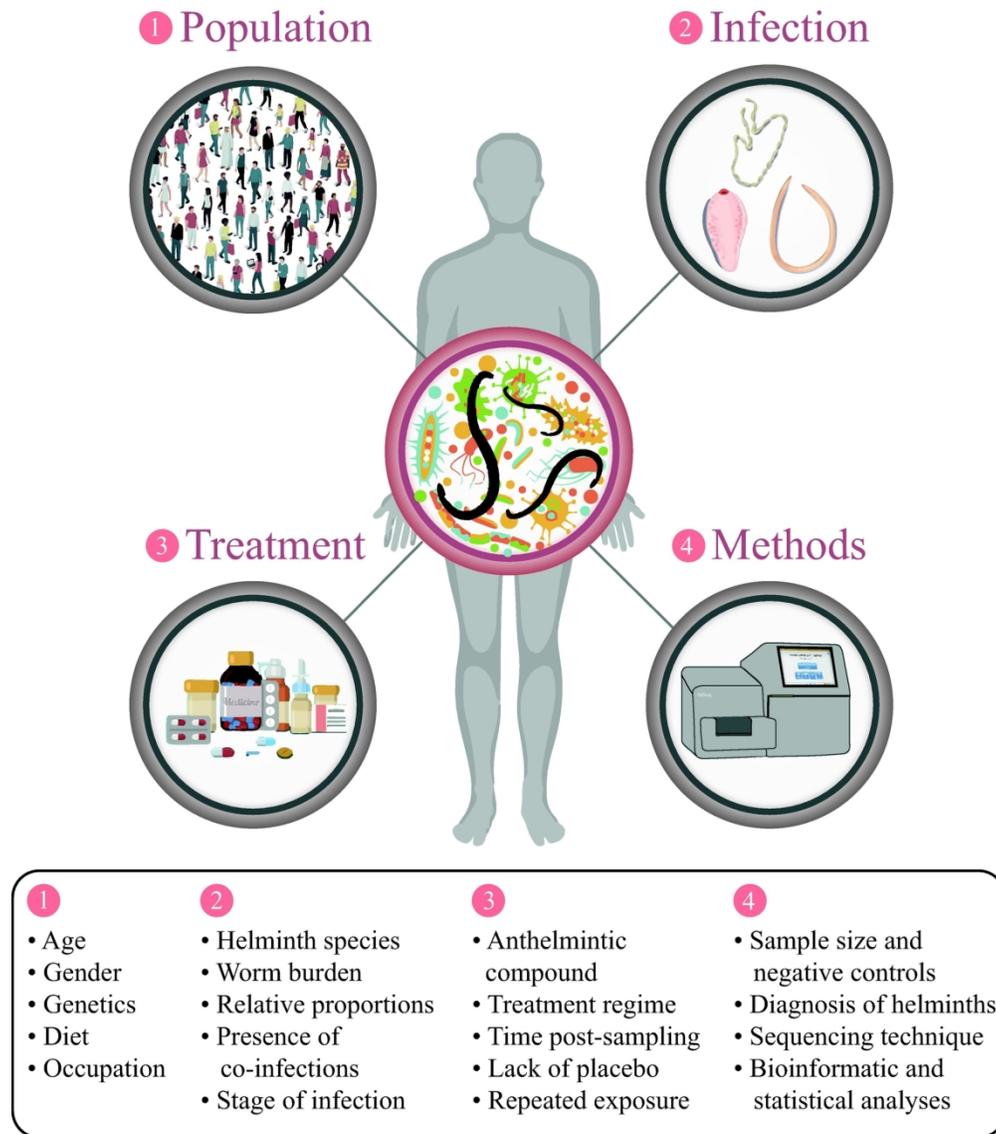


Figure 1. Sources of variation and confounding factors potentially impacting the outcome of studies of human-helminth-gut microbiota interactions in helminth-endemic regions.

158x180mm (300 x 300 DPI)

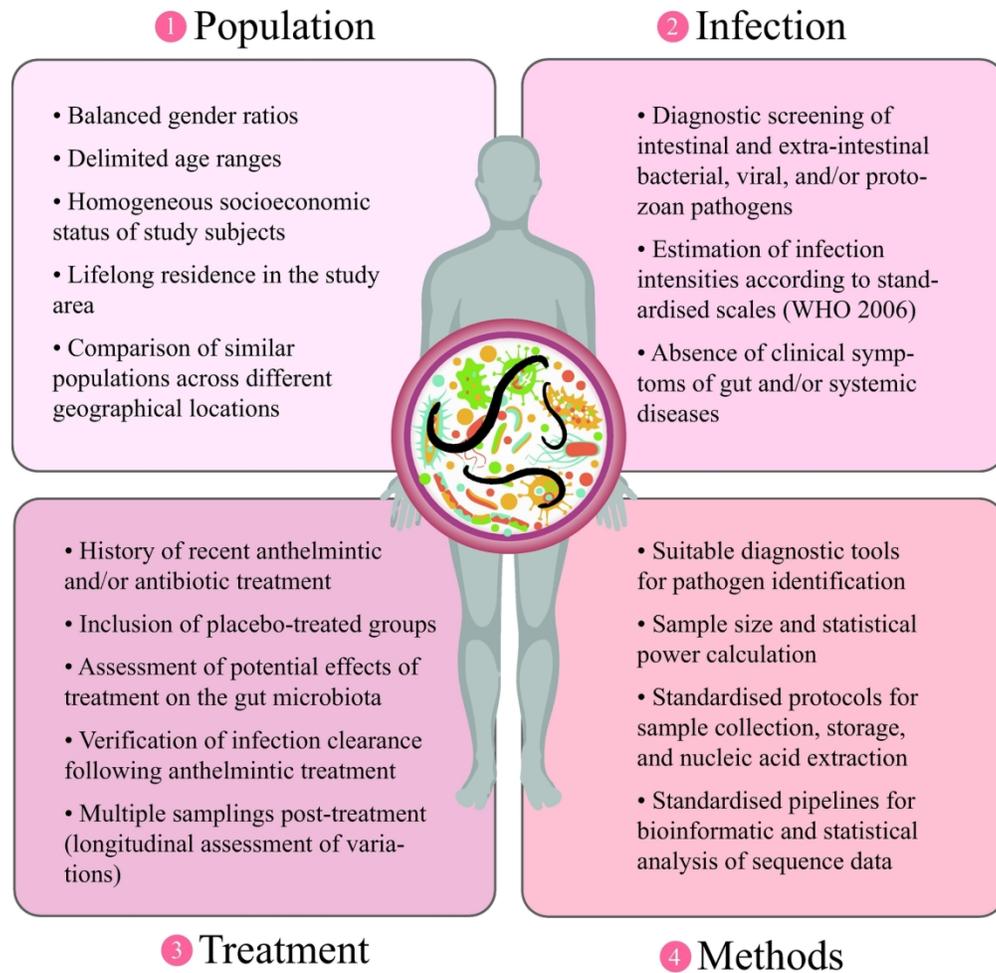


Figure 2. Proposed approaches aimed at reducing the methodological sources of variation surrounding investigations of human-helminth-gut microbiota interactions.

163x164mm (300 x 300 DPI)

Invited review

1
2
3 **Helminths and microbes within the vertebrate gut –**
4 **not all studies are created equal**

5
6 Alba Cortés¹, Laura E. Peachey^{1,2}, Timothy P. Jenkins¹, Riccardo Scotti¹ and Cinzia Cantacessi¹

7
8 ¹*Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES,*
9 *Cambridge, United Kingdom*

10 ²*Bristol Veterinary School, Faculty of Health Sciences, University of Bristol, Langford House,*
11 *Langford, BS40 5DU, Bristol, United Kingdom*

12
13
14 **Running title:** Helminth-microbiota interactions in the human gut

15
16
17 **Corresponding author:**

18 Cinzia Cantacessi

19 *Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES,*
20 *Cambridge, United Kingdom*

21 Tel. +44 (0) 1223 760541

22 Fax. +44 (0)1223 337610

23 E-mail: cc779@cam.ac.uk

24
25 ~~4,648421~~ words
26
27
28

29 SUMMARY

30 The multifaceted interactions occurring between gastrointestinal (GI) parasitic helminths and the
31 host gut microbiota are emerging as a key area of study within the broader research domain of
32 host-pathogen relationships. Over the past few years, a wealth of investigations has demonstrated
33 that GI helminths interact with the host gut flora, and that such interactions result in modifications
34 of the host immune and metabolic statuses. Nevertheless, whilst selected changes in gut microbial
35 composition are consistently observed in response to GI helminth infections across several host-
36 parasite systems, research in this area to date is largely characterised by inconsistent findings.
37 These discrepancies are particularly evident when data from studies of GI helminth-microbiota
38 interactions conducted in humans from parasite-endemic regions are compared. In this review,
39 we provide an overview of the main sources of variance that affect investigations on human-
40 helminth-gut microbiota interactions and propose a series of methodological approaches that,
41 whilst ~~accounting for taking into account~~ the inevitable constraints of human fieldwork, are aimed
42 at minimising confounding factors and draw biologically meaningful interpretations from highly
43 variable datasets.

44

45 1. INTRODUCTION

46 A plethora of experimental evidence supports a key role of infections by gastrointestinal (GI)
47 helminth parasites in shaping the composition of the vertebrate gut microbiota, with significant
48 implications for local and systemic host immunity (reviewed by Brosschot and Reynolds, 2018).
49 For instance, recent studies have partly attributed the parasite-associated qualitative and/or
50 quantitative alterations to host GI microbial profiles to the ability of GI helminths to stimulate the
51 initiate initial the onset of T-regulatory (Treg) immune mechanisms responses, which result in
52 down-regulation of inflammatory responses and establishment of chronic infections, to
53 helminth parasite-associated qualitative and/or quantitative alterations to GI microbial profiles the
54 ability to initiate the onset of T-regulatory (Treg) immune mechanisms, that result in down-
55 regulation of inflammatory responses and establishment of chronic infections (cf. Cantacessi *et*
56 *al.* 2014; Reynolds *et al.* 2014; Giacomini *et al.* 2015, 2016; Zaiss *et al.* 2016). On the other hand,
57 other studies have reported associations between acute helminth infections and gut microbiome
58 microbiota imbalances (= dysbiosis) characterised by that involve significant expansion of
59 populations of putative pro-inflammatory bacteria (e.g. Rausch *et al.* 2013; Jenkins *et al.* 2018a;
60 Schneeberger *et al.* 2018a); these observations have, thus lending lent credit to the hypothesis that
61 helminth-associated alterations of gut microbiota composition may lead to both localised and
62 systemic consequences for the host organism, that includeing immunopathology and (e.g. Rausch
63 *et al.* 2013; Jenkins *et al.* 2018a; Schneeberger *et al.* 2018a), and as well as exacerbated
64 malnutrition in at-risk subjects from parasite-endemic areas (reviewed by Glendinning *et al.* 2014;
65 Houlden *et al.* 2015; Cattadori *et al.* 2016).

66 Over the past decade, newly acquired knowledge of the impact that GI helminth infections exert
67 on the vertebrate gut microbiome composition and metabolism has contributed to a better
68 understanding of parasite systems biology and host-pathogen interactions (reviewed by Peachey
69 *et al.* 2017; Leung *et al.* 2018; Rapin and Harris *et al.* 2018), and has been proposed as a first step
70 towards the identification and development of novel strategies of parasite control based on the
71 targeted manipulation of the host gut microbiota (cf. Peachey *et al.* 2017). Nevertheless, for
72 humans in particular, progress in this field of research is greatly impaired by the impact of several

73 confounding factors that inevitably affect studies conducted in naturally infected individuals
74 (Mutapi, 2015; Chabé *et al.* 2017). In this review, we summarise current knowledge of GI
75 helminth-microbiome interactions in humans under natural conditions of infection, identify
76 similarities and differences between datasets and provide an overview of the confounding factors
77 that may affect the interpretation of findings.

78 2. HUMAN-HELMINTH-GUT MICROBIOTA INTERACTIONS IN REAL-WORLD 79 SCENARIOS

80 In endemic areas for helminthiases, the vast majority of infected individuals harbour multiple
81 helminth species, often occupying different niches of the host organism (Hotez *et al.* 2010).
82 Whilst polyparasitism is often regarded as a major confounding factor in investigations of
83 parasite-microbiota interactions conducted in humans under natural conditions of infection
84 (Cooper *et al.* 2013; Jenkins *et al.* 2017; Martin *et al.* 2018; Rosa *et al.* 2018), findings from these
85 studies are key to assess^{ing} the impact that GI helminths exert on gut microbiota homeostasis in
86 a ‘real-world’ scenario. Nevertheless, several factors should be considered when interpreting
87 results obtained from individuals infected by multiple helminth species. First, anthropometric
88 (e.g. age and gender) and anthropologic variables (e.g. ethnicity, diet and occupation) are well
89 known to profoundly impact the ‘baseline’ composition of the human gut microbiota^{tame} (Sekirov
90 *et al.* 2010; Yatsunenکو *et al.* 2012) (cf. Fig. 1); therefore, the enrolment of large cohorts of
91 individuals is often necessary in order to achieve sufficient statistical power and avoid
92 uninformative and/or misleading results (Kelly *et al.* 2015). However, in many studies, the
93 number of individuals enrolled and samples analysed is inevitably dictated by logistical and
94 financial constraints. In these instances, population-related variables that impact gut microbiota
95 composition may contribute substantially to inconsistencies among findings from different studies
96 (cf. Fig. 1). For instance, a negative association between colonisation by the whipworm *Trichuris*
97 *trichiura* and the abundance of bacteria belonging to the genus *Prevotella* in the faeces of infected
98 individuals has been reported in two separate studies conducted in Malaysia (Lee *et al.* 2014;
99 Ranaman *et al.* 2016), while other studies conducted in Ecuador, and Liberia and Indonesia,

100 respectively, have failed to identify significant variations in faecal populations of *Prevotella* in
101 individuals either solely infected by *T. trichiura* or co-infected with other species of soil-
102 transmitted helminths (STHs) (Cooper *et al.* 2013; Martin *et al.* 2015; Rosa *et al.* 2018).

103 In addition, whilst Rosa and co-authors (2018) detected several distinctive features in the gut
104 microbial profiles of helminth-harboring individuals that were specifically associated to single
105 infections with the hookworm *Necator americanus*, the roundworm *Ascaris lumbricoides* or *T.*
106 *trichiura*, such features were inconsistent between two independent cohorts of helminth-infected
107 volunteers from Liberia and Indonesia, ~~respectively~~; this discrepancy suggests that other yet
108 undetermined environmental factors may contribute to qualitative and quantitative alterations of
109 the gut microbial profiles of helminth-infected individuals from different geographical areas. In
110 contrast, an association between the abundance of selected bacterial taxa and infections by one or
111 more STHs could be consistently detected in samples from both Liberian and Indonesian cohorts
112 (Rosa *et al.* 2018). These taxa included bacteria belonging to the genera *Olsenella* and
113 *Allobaculum*, which were expanded in the gut microbiota of helminth-infected individuals when
114 compared to that of uninfected controls. To the best of our knowledge, the study by Rosa *et al.*
115 (2018) was the first to report a link between infections by STHs and the abundance of these
116 bacterial genera in the human gut. Interestingly, in mice suffering from metabolic syndrome,
117 administration of probiotics was followed by expansion of populations of *Olsenella* and/or
118 *Allobaculum*, and a reduction in systemic and/or local gut inflammatory responses (Wang *et al.*
119 2015). Moreover, *Allobaculum* spp. are putative producers of anti-inflammatory short-chain fatty
120 acids (Greetham *et al.* 2004), and are severely reduced in the gut of mice genetically predisposed
121 to spontaneous colitis (Pérez-Muñoz *et al.* 2014). This knowledge led Rosa *et al.* (2018) to
122 hypothesize that these bacteria may play a yet undetermined role in the anti-inflammatory
123 properties of parasitic helminths, ~~and reinforce the proposition that the interactions between thus~~
124 ~~underpinning the general idea that hosts, -parasites and -gut microbiota are interactions are~~
125 ~~multidirectional and should be approached in from a holistic perspective manner (e.g. Cortés *et*~~
126 ~~al. 2018; Leung *et al.* 2018). Interestingly, in contrast to evidence acquired in human hosts, a~~

127 negative association between the genus *Allobaculum* and colonisation by GI helminths has been
128 observed in [a mouse model of chronic trichuriasis](#) ~~mice chronically infected with *T. muris*~~ (Holm
129 *et al.* 2015), ~~in which is featured by a dominant Th1-mediated immune responses are dominant~~
130 ~~(reviewed by Cliffe and Grencis, 2004), as well as in mice and~~ with patent infection by the blood
131 fluke *Schistosoma mansoni* (Jenkins *et al.* 2018a), ~~in which migrating eggs are responsible for the~~
132 ~~onset of marked Th2-mediated inflammatory responses are elicited to migrating eggs~~ (reviewed
133 ~~by Pearce and MacDonald, 2002). The immune-molecular mechanisms through-via which~~
134 ~~members of the genus *Allobaculum* may regulate local and systemic inflammation are yet-to-be~~
135 ~~elucidated still unclear~~ (Greetham *et al.* 2004; Pérez-Muñoz *et al.* 2014; Wang *et al.* 2015).
136 ~~Nonetheless, current data experimental evidence on showing concomitant reductions in~~
137 ~~populations of *Allobaculum* and alongside helminth-associated gut inflammation supports seems~~
138 ~~consistent with the hypothesis of raised by Rosa *et al.* (2018); in the future, suggesting that~~
139 ~~laboratory rodent models of GI helminthiasis helminth infections whose gut microbiota is~~
140 ~~deprived of, and subsequently recolonised with, the genus *Allobaculum* could be exploited to~~
141 ~~investigate the potential involvement of these bacteria in the parasite-mediated~~
142 ~~immunomodulation mediated by helminth parasites (e.g. via exogenous recolonization with~~
143 ~~*Allobaculum* spp.).~~ Notably, both models of helminth infection are characterised by the occurrence
144 of severe intestinal inflammation involving different populations of T CD4+ cells (i.e. Th1 and
145 Th2, respectively; Pearce and MacDonald, 2002; Cliffe and Grencis, 2004), and therefore, the
146 observed reduction in populations of *Allobaculum* in these systems supports the immune
147 regulatory role for this bacterial genus.

148 Beside the intrinsic variability of the human gut microbiota, studies conducted under natural
149 conditions of helminth colonisation are likely to be affected by factors linked to the different
150 combinations of infecting species and their relative abundances. For instance, in a study
151 conducted in a cohort of Ecuadorian children, the specific features detected in the gut microbial
152 profiles of subjects co-infected with *T. trichiura* and *A. lumbricoides* could not be identified in
153 the microbiota of *Trichuris*-only infected individuals (Cooper *et al.* 2013). Similarly, selected

154 microbial features that were observed in studies conducted in human volunteers with mono-
155 specific infections with, for instance, *A. lumbricoides*, could not be detected in the gut microbiota
156 of subjects harbouring the same parasite alongside other helminth species (e.g. *T. trichiura* and
157 *N. americanus*) (Rosa *et al.* 2018), thus suggesting that a complex interplay exists between the
158 host gut and its macro- and microbiota, that might be difficult to replicate in experimental settings.
159 Furthermore, current evidence obtained from animal models of helminth infections indicates that
160 worm burdens can impact the nature and/or the magnitude of parasite-associated alterations in gut
161 microbial composition (Wu *et al.* 2012; Peachey *et al.* 2018); ~~nevertheless~~ Nevertheless, such
162 evidence is not yet available for human infections, in which whose burdens parasite burdens in
163 endemic areas may range from low to very high due to overdispersion of parasite loads in endemic
164 areas (Barbour and Kafetzaki, 1991; Churcher *et al.* 2005) and, therefore, are likely to be an
165 important confounding factor for studies of parasite-microbiota interactions in naturally infected
166 individuals.

167 Another frequent constraint of investigations conducted in cohorts of human subjects with natural
168 helminth infections is the limited availability of ‘genuine’ negative controls, i.e. individuals from
169 the same communities of parasite-infected subjects who lack previous exposure to infections by
170 parasitic helminths. Instead, individuals with no evidence of patent helminth infections are
171 inevitably enrolled as control subjects (e.g. Cooper *et al.* 2013; Lee *et al.* 2014; Jenkins *et al.*
172 2017; Rosa *et al.* 2018); nevertheless, studies in helminth-infected individuals subjected to
173 anthelmintic treatment, as well as in primates and pigs exposed to *Trichuris* spp., have shown that
174 parasite-associated alterations in ~~the~~ gut microbial communities can persist, at least part~~ly~~ially, in
175 absence of active infections (Broadhurst *et al.* 2012; Wu *et al.* 2012; Cooper *et al.* 2013; Kay *et*
176 *al.* 2015; Schneeberger *et al.* 2018a). These data call for caution when interpreting differences
177 between the gut microbial profiles of helminth-infected and uninfected volunteers from the same
178 communities. In addition, patent infections are often diagnosed using stool-based microscopic
179 methods, that are known for their relatively low sensitivity and that may yield false negative
180 results, e.g. in case of intermittent shedding of eggs and/or larvae (O’Connell and Nutman, 2016).

181 Recently, Rosa *et al.* (2018) used quantitative real-time PCR to diagnose STH infections in
182 individuals subjected to gut microbiota profiling, indicating that this technique may represent
183 a robust and sensitive alternative to microscopic methods, since it provides users with the ability
184 to semi-quantify burdens of different helminth species from minute amounts of DNA template.
185 However, in spite of their higher sensitivity, molecular methods rely on the use of primers that
186 selectively target the parasite species of interest, thus impairing the simultaneous detection of
187 potential (asymptomatic or subclinical) co-infections with other helminth and/or non-helminth
188 pathogens (O'Connell and Nutman, 2016). Indeed, the impact of protozoa on the gut microbial
189 diversity and composition has been clearly demonstrated in humans and other vertebrates
190 (reviewed by Chabé *et al.* 2017; Stensvold and van der Giezen, 2018). Furthermore, a recent study
191 conducted in a cohort of Colombian schoolchildren reported common features in the faecal
192 microbial composition of subjects co-infected with helminths and protozoans and mono-
193 parasitized with the flagellate *Giardia intestinalis* compared to uninfected individuals (Toro-
194 Londono *et al.* 2019). Whilst the mechanisms *via* which each group of parasites alters the host
195 gut flora, as well as the nature of such alterations, are yet to be determined, these findings support
196 the need to conduct additional diagnostic tests on stool samples from helminth-infected cohorts,
197 as well as the corresponding uninfected subjects, in order to rule out the influence of concomitant
198 bacterial, viral and/or protozoan infections that may be responsible for the changing gut microbial
199 profiles of these individuals (cf. Chabé *et al.* 2017).

200 Nevertheless, in spite of the several confounding factors outlined above (cf. Fig. 1), observational
201 studies in helminth endemic areas have proven useful for the identification of significant
202 associations between parasite colonisation and the gut microbial profiles of humans under natural
203 conditions of infection. Importantly, studies conducted in these communities provide excellent
204 opportunities to evaluate the effect(s) that parasite removal (e.g. via the administration of broad-
205 spectrum anthelmintics) exert(s) on the gut microbiota of previously infected individuals, thus
206 contributing cues to understand the causality of helminth-microbiota relationships.

207 3. IMPACT OF DEWORMING ON THE HUMAN GUT MICROBIOTA

208 The implementation of mass drug administration programmes in endemic areas for STHs and
209 schistosomiasis offers opportunities to elucidate potential mechanisms *via* which parasitic
210 helminths modulate the host gut microbiota. For instance, qualitative and quantitative changes in
211 gut microbial profiles that are caused by direct interactions between parasites and gut bacteria
212 may be expected to rapidly reverse following parasite removal, whilst long-lasting alterations are
213 likely to result from indirect interplay mediated by the host immune system (Houlden *et al.* 2015;
214 Su *et al.* 2018). Nevertheless, such investigations are also generally constrained by the presence
215 of several confounding factors that include not only the host- and parasite-dependent variables
216 outlined above, but also variations linked to the use of different drugs and treatment regimes
217 (Schneeberger *et al.* 2018b), as well as time of sampling post-anthelmintic treatment (Houlden *et*
218 *al.* 2015) (Fig. 1). The latter in particular may profoundly affect findings from these studies, as
219 the presence of tissue lesions caused by e.g. parasite feeding activity and location (e.g. blood-
220 feeders *vs.* non blood-feeders and luminal *vs.* tissue dwellers) are likely to influence the timespan
221 between helminth removal and microbiome recovery (reviewed by Leung *et al.* 2018). Moreover,
222 for ethical reasons, data from these experiments is often biased by the lack of placebo-treated
223 control groups. These limitations may be at least partially responsible for the differences between
224 findings from studies aimed ~~to elucidate~~ elucidating the effect of deworming on the gut
225 microbiota of helminth-infected volunteers; notwithstanding, it is worth noting that, in instances
226 where deworming-associated changes in human gut microbial profiles were detected, these were
227 generally moderate (Ramanan *et al.* 2016; Martin *et al.* 2018; Schneeberger *et al.* 2018b).

228 Consistent with this, a recent study conducted on faecal samples collected from a rural community
229 in Indonesia reported that the composition of the gut microbiota of individuals repeatedly
230 treated with either albendazole or placebo (for 21 months) resembled that of samples collected
231 from the same subjects prior to treatment, rather than that of uninfected controls (Rosa *et al.*
232 2018). Moreover, a parallel investigation conducted on the same cohort of individuals detected
233 reduced populations of *Prevotella* in albendazole-treated subjects in which complete deworming
234 did not occur, compared to placebo-treated individuals with patent helminth infections (Martin *et*

235 *al.* 2018). Intriguingly, failure of albendazole treatment was accompanied by a dominance of *T.*
236 *trichiura* (over other helminth species) in these subjects, while placebo-treated individuals
237 maintained a diverse macrobiota (i.e. multiple helminth infections); hence, differences in the
238 composition of the GI macrobiota (i.e. species present and their relative abundances) between
239 albendazole- and placebo-treated individuals could account for variations in the composition of
240 the intestinal microflora of these subjects (Martin *et al.* 2018). Significant associations between
241 colonisation by *T. trichiura* and *Prevotella* abundance were not observed in the Indonesian cohort
242 (Martin *et al.* 2018; Rosa *et al.* 2018). However, negative associations between whipworm
243 infections and *Prevotella* abundance had been detected previously in two independent studies
244 conducted in Malaysia (Lee *et al.* 2014; Ramanan *et al.* 2016). In particular, Ramanan and co-
245 authors (2016) observed that, following albendazole treatment, expansion of *Prevotella*
246 populations in the human faecal microbiota was related to reduced *T. trichiura* faecal egg counts.
247 In contrast, no significant associations between helminth infection and abundance of bacteria
248 belonging to the genus *Prevotella* was reported in a study investigating the impact of parasite
249 colonisation and [effective-successful treatment with a combination of](#) albendazole and ivermectin
250 [treatment](#) on the faecal microbial profiles of a cohort of *Trichuris*-infected children from Ecuador
251 (Cooper *et al.* 2013), nor in a group of helminth-infected adults from Sri Lanka treated with
252 pyrantel pamoate (Jenkins *et al.* 2017). Similarly, no qualitative or quantitative changes to faecal
253 microbial composition were observed in two cohorts of schoolchildren from Côte d'Ivoire and
254 Zimbabwe infected by *S. mansoni* and *S. haematobium*, respectively, following treatment with
255 praziquantel (Kay *et al.* 2014; Schneeberger *et al.* 2018a). However, successful elimination of *S.*
256 *mansoni* was associated with a higher abundance of *Fusobacterium* spp. pre-treatment, as well as
257 24 hrs post-treatment (Schneeberger *et al.* 2018a).

258 Whilst drug administration in endemic regions may result in effective elimination of helminth
259 infections, potential co-infecting protozoan parasites are not susceptible to anthelmintic
260 treatment; this, together with the sub-standard hygienic and sanitary conditions that generally
261 characterise these areas and that result in continuous re-exposure to infective helminth

262 developmental stages (Campbell *et al.* 2018), impairs the full assessment of the consequences of
263 helminth removal on the composition of the human gut microbiota. To the best of our knowledge,
264 thus far, a single study has investigated the effects of chronic infections by a GI helminth,
265 *Strongyloides stercoralis*, and anthelmintic treatment on the composition of the faecal
266 microbiota and metabolome of humans from a non-endemic area of Europe, where parasite
267 transmission had been interrupted (Jenkins *et al.* 2018b). Treatment with ivermectin resulted in
268 compositional changes of the faecal microbiota (analysed 6 months post-treatment), which
269 partially resembled that of uninfected control subjects (Jenkins *et al.* 2018b); in particular, alpha
270 diversity [= a measure of the number of bacterial species present in a given microbial community
271 (richness) and their relative abundance (evenness)] was reduced in the microbiota of ~~the former~~
272 ~~group of dewormed~~ individuals ~~post-treatment~~ (although statistical significance was not achieved)
273 and accompanied by expanded populations of potentially pathogenic bacteria (Jenkins *et al.*
274 2018b). In addition, the faecal metabolic profiles obtained from samples collected post-ivermectin
275 treatment ~~shared features with both appeared to fall somewhere in between~~ those obtained from
276 samples collected pre-treatment ~~as well as from and from~~ uninfected controls (Jenkins *et al.*
277 ~~2018b); this observation led Jenkins et al. (2018b), to hypothesise that, thus supporting the notion~~
278 ~~that, following parasite removal and over time, suggesting a (direct and/or indirect) effect of~~
279 ~~parasite infection and removal on~~ both gut microbiota and metabolome ~~may revert to the~~
280 ~~original a pre-infection state~~. Multiple factors, including but not limited to those outlined above,
281 may contribute to the discrepancies observed between the findings from this work and those that
282 reported no or minor effects of anthelmintic treatment on the gut microbiome of helminth-infected
283 humans (Cooper *et al.* 2013; Ramanan *et al.* 2016; Martin *et al.* 2018; Rosa *et al.* 2018;
284 Schneeberger *et al.* 2018a,b).

285 Despite the limitations outlined above, studies of GI helminth-microbiota relationships conducted
286 in endemic areas for helminthiasis have provided repeated evidence of the perturbations that
287 parasites and anthelmintic treatment exert on the equilibrium of resident populations of gut
288 bacteria and on gut homeostasis. However, the identification of common signatures across studies

289 remains key to designing future experiments, e.g. in animal models of helminth infections, that
290 may assist the elucidation of the mechanisms that underpin the interactions between GI helminths,
291 the gut microbiota and the host immune system.

292 4. DO COMMON SIGNATURES EXIST ACROSS STUDIES OF HOST-HELMINTH- 293 MICROBIOTA INTERACTIONS?

294 The identification of gut microbial signatures that occur reproducibly across several host-GI
295 helminth systems is crucial for designing novel anti-helminth intervention strategies based on the
296 manipulation of the gut microbiota (Peachey *et al.* 2017). Studies conducted in animal models of
297 helminth infections are expected to assist the identification of such signatures, as well as the direct
298 (i.e. parasite-mediated) and/or indirect (i.e. immune-mediated) mechanisms that govern helminth-
299 microbiota interactions (Cortés *et al.* 2018); nevertheless, the inconsistencies that characterise
300 studies of helminth-microbiota relationships published to date make such a task highly
301 challenging. Indeed, for patterns to be identified, fluctuations in selected populations of gut
302 microbes must be interpreted in light of the physical and immunological alterations of the mucosal
303 environment in which such alterations occur (Leung *et al.* 2018). For instance, expanded
304 populations of *Lactobacillaceae* have been repeatedly detected following infection with several
305 species of parasitic helminths in several host species (Reynolds *et al.* 2014; Duarte *et al.* 2015;
306 Holm *et al.* 2015; Houlden *et al.* 2015; Cattadori *et al.* 2016; Jenkins *et al.* 2018a; Kim *et al.*
307 2018), and could thus be considered as a ‘consistent alteration’ in gut microbiota composition
308 upon helminth colonisation. However, key differences exist between host-parasite pairs
309 investigated in the studies that have reported such an outcome. Indeed, whilst populations of
310 *Lactobacillaceae* promote regulatory responses in mice infected by *Heligmosomoides polygyrus*
311 *bakeri* (Reynolds *et al.* 2014), a lack of correlation between *Lactobacillaceae* abundance and Treg
312 populations has been observed in other host-parasites systems, such as mice chronically infected
313 with *T. muris* and rabbits infected with *Trichostrongylus retortaeformis*, in which the expansion
314 of populations of gut *Lactobacillaceae* upon helminth infection occurs in an environment
315 dominated by Th1-mediated immune responses (Holm *et al.* 2015; Houlden *et al.* 2015; Cattadori

316 *et al.* 2016). These differences suggest that alternative mechanisms may regulate the
317 differentiation and development of adaptive immune responses in each host-parasite system
318 (Houlden *et al.* 2015), and thus that similar alterations in gut microbiota composition may result
319 in different consequences that are dependent on the microenvironment where these changes occur.
320 Notwithstanding, the interactions between hosts, helminths and the gut microbiota are likely
321 multifaceted and multidirectional, and therefore the potential consequences that selected
322 compositional changes in gut microbiota exert on host homeostasis are only one aspect of these
323 complex interplay. For instance, a common yet undetermined mechanism may determine the
324 expansion of *Lactobacillaceae* in the gut of helminth-infected hosts.

325 On the other hand, apparent 'contradictory' findings across studies may result from fundamental
326 differences between gut compartments under investigation. For instance, *Prevotella* spp. was
327 expanded in the abomasum and faeces of sheep infected by abomasal trichostrongyles (i.e.
328 *Haemonchus contortus* and *Teladorsagia circumcincta*; Li *et al.* 2016; Cortés *et al.* in
329 preparation), whilst the same taxa were reduced in the faeces of a range of host species, including
330 mice, humans and horses, infected by nematodes residing in the large intestine, i.e. *Trichuris* spp.
331 and cyathostomins, respectively (Lee *et al.* 2014; Houlden *et al.* 2015; Peachey *et al.* submitted).
332 It must be noted, however, that whilst increased abomasal pH favours *Prevotella* overgrowth in
333 the abomasum (De Nardi *et al.* 2016; Li *et al.* 2016), the same taxa are likely to be exposed to a
334 dramatically different microenvironment in the large intestine that may determine the contraction
335 of these bacterial taxagroups. In addition, given the functional dissimilarities between the
336 abomasal and colonic microbiota, such alterations are expected to result in fundamentally
337 different outcomes for the homeostasis of each of these gut compartments (Ley *et al.* 2008), and
338 hence comparisons are, in our opinion, unwarranted.

339 In parallel to species of bacteria with functions that may vary depending on the gut compartment,
340 multiple taxa share the same functions in different microenvironments (Lozupone *et al.* 2012);
341 therefore, it is plausible that, even though inconsistencies are detected across studies, these may
342 result in similar functional alterations in the host-parasite pairs being compared. For instance,

343 recent studies in mouse and humans infected with *S. mansoni* have reported the expansion of
344 different genera of bacteria with pro-inflammatory functions in the gut microbiota of the
345 respective hosts (Jenkins *et al.* 2018a; Schneeberger *et al.* 2018a). These observations lend credit
346 to the hypothesis that the functional role of the gut microbiota in helminth infections could be far
347 less 'diverse' than the taxonomic associations reported thus far. For this hypothesis to be
348 confirmed or confuted, a better understanding of the function(s) of each bacterial taxon inhabiting
349 the different gut compartments in a range of host species is needed. To this aim, the integration
350 of metagenomic, metabolomic and metatranscriptomic technologies, alongside traditional
351 microbiology and microscopy techniques, may assist to achieve a holistic picture of the impact of
352 GI helminth infections on the functions of the human gut microbiota, and its significance for
353 disease pathophysiology and overall host health (Wang *et al.* 2015).

354 5. CURRENT NEEDS AND FUTURE DIRECTIONS

355 Understanding the complex interactions between GI helminths and their vertebrate hosts is pivotal
356 for advancing our knowledge of the fundamental biology of these parasites and the diseases they
357 cause (see Peachey *et al.* 2017; Leung *et al.* 2018; Rapin and Harris *et al.* 2018 for reviews).
358 Whilst the role of the gut microbiota in host-parasite relationships has long been overlooked,
359 current knowledge of the key roles that resident bacteria play in host health and disease, together
360 with recent technical advancements for microbiota profiling, have boosted research in this area.
361 This is currently leading to increasing evidence of ~~an active involvement of the gut microbiota in~~
362 ~~the immunopathology of GI helminth infections (e.g. Rausch *et al.* 2013; Jenkins *et al.* 2018a;~~
363 ~~Schneeberger *et al.* 2018a). Furthermore, several studies support~~ a role for the gut microbiota in
364 the immune regulatory properties of helminth parasites (Cantacessi *et al.* 2014; Reynolds *et al.*
365 2014; Giacomini *et al.* 2015, 2016; Zaiss *et al.* 2016). ~~Furthermore~~In addition, ~~data collected to~~
366 ~~date points towards a likely role~~ ~~whether certain members of the gut microflora in are actively~~
367 ~~involved in the immunopathology of particular selected GI helminth infections that awaits~~
368 ~~experimental validation is a currently outstanding question that awaits for a response. Indeed,~~
369 ~~whilst~~ Trying to untangle the relevance of particular fluctuations of specific bacterial taxa on

370 infection outcome is challenging; nevertheless, currently available data suggest that low-
371 intensity, long-term helminth infections are commonly linked to high microbial diversity and
372 predominance of bacteria typically associated with gut health.; Conversely, high-intensity, acute
373 infections are often associated to gut dysbiosis, characterised by reduced alpha diversity and an
374 increase in pro-inflammatory and often opportunistic pathogens (Peachey *et al.* 2017). However,
375 for this knowledge to be exploited in translational studies, further investigations in both natural
376 and experimental settings are needed to distinguish spurious results from genuine helminth-
377 microbiota associations (Peachey *et al.* 2017), and mechanistic studies in animal models of
378 helminth infections are necessary to dissect the causality of these relationships (cf. Cortés *et al.*
379 2018). Importantly, minimising variations between studies is crucial to warrant meaningful
380 comparisons between datasets.

381 Whilst reducing the variability amongst samples collected from naturally helminth-infected
382 humans may be difficult to achieve, the enormous impact that differences in technical and
383 experimental approaches (from sample collection to bioinformatics and biostatistical analysis)
384 exert on the overall variation detected across studies can be reduced (Figs. 1 and 2; Lindgreen *et*
385 *al.* 2017; Costea *et al.* 2017; Golob *et al.* 2017). In particular, a range of bioinformatics pipelines
386 are available for the analysis of high-throughput amplicon and metagenomics sequence datasets
387 that include, e.g., different sequence-processing tools and reference databases for sequence
388 annotation that could yield slightly different results (Lindgreen *et al.* 2017; Golob *et al.* 2017).

389 For instance, the use of validated open microbiome analysis packages such Multiplexed Analysis
390 of Projections by Sequencing (MAPseq) (Matias Rodrigues *et al.* 2017) or QIIME2
391 (<https://qiime2.org/>) taxonomy classification of 16S amplicon datasets, for instance, current
392 trends indicate that optimised approaches should rely on open microbiome analysis packages such
393 Multiplexed Analysis of Projections by Sequencing (MAPseq) (Matias Rodrigues *et al.* 2017) or
394 QIIME2 (<https://qiime2.org/>), which have proven fast, accurate and specific in predicting
395 taxonomic affiliations, may assist accurate taxonomic classifications of bacterial 16S rRNA
396 amplicon datasets; similarly, sequence annotation should rely on the use of and the usage of

397 ~~comprehensive, as well as~~ regularly updated reference databases. Amongst these, ~~, e.g.~~ SILVA
398 (<https://www.arb-silva.de/>) (Quast *et al.* 2013), ~~that enables a~~ sensitive annotations of bacterial
399 rRNA sequence data (Almeida *et al.* 2018). Thus, ~~the~~ use of such standardized analysis
400 workflows and ~~continuously updated~~ reference databases for sequence annotation might prove
401 extremely useful to increase consistency across studies and enable researchers to identify common
402 and/or unique features between the gut microbiota of different host-parasite systems which, in
403 turn, might assist to better understand the mechanisms that regulate helminth-microbiota
404 relationships.

405 The consequences that elucidating such mechanisms may exert on future strategies of parasite
406 control are two-fold. First, disentangling the potential contribution of the gut flora to the
407 pathogenesis of the infection is necessary in order to discover and develop new strategies to
408 contrast helminth-associated pathology. Second, understanding the microbiota-dependent
409 mechanisms by which parasitic helminths are able to modulate host immune responses and
410 suppress inflammation may assist the discovery of novel immune-regulatory therapeutics against
411 chronic inflammatory disorders of the GI tract that may act in synergy with helminth-based
412 therapy (see Peachey *et al.* 2017 and Rapin and Harris, 2018 for reviews). However, in order for
413 this new knowledge to be fully exploited in translational research, further studies that thoroughly
414 consider inclusion/exclusion criteria for the selection of participants, include appropriate controls,
415 and follow standardised experimental and data analysis protocols; are necessary, thus allowing and
416 will allow to disentangle the potential influence of parasite-, drug- and/or population-dependent
417 variables in each setting (Fig. 2), ~~are necessary~~.

418

419

420 ACKNOWLEDGEMENTS

421 The authors would like to thank Professor R. Stephen Phillips for helpful suggestions on the draft
422 manuscript.

423 FINANCIAL SUPPORT

424 AC is supported by a postdoctoral fellowship from Fundación Alfonso Martín Escudero (Madrid,
425 Spain). LEP is supported by funding from the Horserace Betting Levy Board (HBLB) and TPJ
426 by scholarships by the Biotechnology and Biological Sciences Research Council (BBSRC) of the
427 United Kingdom. Research in the CC laboratory is supported by grants by the Royal Society and
428 the Isaac Newton Trust.

429

For Peer Review

430 REFERENCES

431 [Almeida A, Mitchell AL, Tarkowska A and Finn RD \(2018\) Benchmarking taxonomic](#)
432 [assignments based on 16S rRNA gene profiling of the microbiota from commonly sampled](#)
433 [environments. *GigaScience* 7, 1-10.](#)

434 [Barbour AD and Kafetzaki M \(1991\) Modeling the overdispersion of parasite loads.](#)
435 [*Mathematical Biosciences* 107, 249-253.](#)

436 **Broadhurst MJ, Ardeshir A, Kanwar B, Mirpuri J, Gundra UM, Leung JM, Wiens KE,**
437 **Vujkovic-Cvijin I, Kim CC, Yarovinsky F, Lerche NW, McCune JM and Loke P (2012)**
438 **Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the**
439 **inflammatory signature and mucosal microbiota of the colon. *PLoS Pathogens* 8, e1003000.**

440 **Brosschot TP and Reynolds LA (2018) The impact of a helminth-modified microbiome on host**
441 **immunity. *Mucosal Immunology* 11, 1039-1046.**

442 **Campbell SJ, Biritwum NK, Woods G, Velleman Y, Fleming F and Stothard JR (2018)**
443 **Tailoring water, sanitation, and hygiene (WASH) targets for soil-transmitted helminthiasis and**
444 **schistosomiasis control. *Trends in Parasitology* 34, 53-63.**

445 **Cantacessi C, Giacomini P, Croese J, Zakrzewski M, Sotillo J, McCann L, Nolan MJ,**
446 **Mitreva M, Krause L and Loukas A (2014) Impact of experimental hookworm infection on the**
447 **human gut microbiota. *The Journal of Infectious Diseases* 210, 1431-1434.**

448 **Cattadori IM, Sebastian A, Hao H, Katani R, Albert I, Eilertson KE, Kapur V, Pathak A**
449 **and Mitchell S (2016) Impact of helminth infections and nutritional constraints on the small**
450 **intestine microbiota. *PLoS One* 11, e0159770.**

451 **Chabé M, Lokmer A and Segurel L (2017) Gut protozoa: friends or foes of the human gut**
452 **microbiota? *Trends in Parasitology* 33, 925-934.**

453 [Churcher TS, Ferguson NM and Basáñez MG \(2005\) Density dependence and](#)
454 [overdispersion in the transmission of helminth parasites. *Parasitology* 131\(Pt 1\), 121-132.](#)

455

- 456 **Cliffe LJ and Grencis RK** (2004) The *Trichuris muris* system: a paradigm of resistance and
457 susceptibility to intestinal nematode infection. *Advances in Parasitology* **57**, 255-307.
- 458 **Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M and Parkhill J** (2013) Patent
459 human infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in
460 the faecal microbiota. *PLoS One* **8**, e76573.
- 461 **Cortés A, Toledo R and Cantacessi C** (2018) Classic models for new perspectives: delving into
462 helminth-microbiota-immune system interactions. *Trends in Parasitology* **34**, 640-654.
- 463 **Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen**
464 **M, Hercog R, Jung FE, Kultima JR, Hayward MR, Coelho LP, Allen-Vercoe E, Bertrand**
465 **L, Blaut M, Brown JRM, Carton T, Cools-Portier S, Daigneault M, Derrien M, Druesne A,**
466 **de Vos WM, Finlay BB, Flint HJ, Guarner F, Hattori M, Heilig H, Luna RA, van Hylckama**
467 **Vlieg J, Junick J, Klymiuk I, Langella P, Le Chatelier E, Mai V, Manichanh C, Martin JC,**
468 **Mery C, Morita H, O'Toole PW, Orvain C, Patil KR, Penders J, Persson S, Pons N, Popova**
469 **M, Salonen A, Saulnier D, Scott KP, Singh B, Slezak K, Veiga P, Versalovic J, Zhao L,**
470 **Zoetendal EG, Ehrlich SD, Dore J and Bork P** (2017) Towards standards for human fecal
471 sample processing in metagenomic studies. *Nature Biotechnology* **35**, 1069-1076.
- 472 **De Nardi R, Marchesini G, Li S, Khafipour E, Plaizier KJ, Ganesella M, Ricci R,**
473 **Andrighetto I and Segato S** (2016) Metagenomic analysis of rumen microbial population in
474 dairy heifers fed a high grain diet supplemented with dicarboxylic acids or polyphenols. *BMC*
475 *Veterinary Research* **12**, 29-016-0653-4.
- 476 **Duarte AM, Jenkins TP, Latrofa MS, Giannelli A, Papadopoulos E, de Carvalho LM, Nolan**
477 **MJ, Otranto D and Cantacessi C** (2016) Helminth infections and gut microbiota - a feline
478 perspective. *Parasites & Vectors* **9**, 625-016-1908-4.
- 479 **Giacomin P, Zakrzewski M, Croese J, Su X, Sotillo J, McCann L, Navarro S, Mitreva M,**
480 **Krause L, Loukas A and Cantacessi C** (2015) Experimental hookworm infection and escalating

- 481 gluten challenges are associated with increased microbial richness in celiac subjects. *Scientific*
482 *Reports* **5**, 13797.
- 483 **Giacomin P, Zakrzewski M, Jenkins TP, Su X, Al-Hallaf R, Croese J, de Vries S, Grant A,**
484 **Mitreva M, Loukas A, Krause L and Cantacessi C** (2016) Changes in duodenal tissue-
485 associated microbiota following hookworm infection and consecutive gluten challenges in
486 humans with coeliac disease. *Scientific Reports* **6**, 36797.
- 487 **Glendinning L, Nausch N, Free A, Taylor DW and Mutapi F** (2014) The microbiota and
488 helminths: sharing the same niche in the human host. *Parasitology* **141**, 1255-1271.
- 489 **Golob JL, Margolis E, Hoffman NG and Fredricks DN** (2017) Evaluating the accuracy of
490 amplicon-based microbiome computational pipelines on simulated human gut microbial
491 communities. *BMC Bioinformatics* **18**, 283-017-1690-0.
- 492 **Greetham HL, Gibson GR, Giffard C, Hippe H, Merkhoffer B, Steiner U, Falsen E and**
493 **Collins MD** (2004) *Allobaculum stercoricanis* gen. nov., sp. nov., isolated from canine
494 feces. *Anaerobe* **10**, 301-307.
- 495 **Holm JB, Sorobetea D, Kiilerich P, Ramayo-Caldas Y, Estelle J, Ma T, Madsen L,**
496 **Kristiansen K and Svensson-Frej M** (2015) Chronic *Trichuris muris* infection decreases
497 diversity of the intestinal microbiota and concomitantly increases the abundance of
498 Lactobacilli. *PLoS One* **10**, e0125495.
- 499 **Hotez PJ, Alvarado M, Basanez MG, Bolliger I, Bourne R, Boussinesq M, Brooker SJ,**
500 **Brown AS, Buckle G, Budke CM, Carabin H, Coffeng LE, Fevre EM, Furst T, Halasa YA,**
501 **Jasrasaria R, Johns NE, Keiser J, King CH, Lozano R, Murdoch ME, O'Hanlon S, Pion SD,**
502 **Pullan RL, Ramaiah KD, Roberts T, Shepard DS, Smith JL, Stolk WA, Undurraga EA,**
503 **Utzinger J, Wang M, Murray CJ and Naghavi M** (2014) The global burden of disease study
504 2010: interpretation and implications for the neglected tropical diseases. *PLoS Neglected Tropical*
505 *Diseases* **8**, e2865.

- 506 **Houlden A, Hayes KS, Bancroft AJ, Worthington JJ, Wang P, Grencis RK and Roberts**
507 **IS** (2015) Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host
508 microbiota and metabolome: effects reversed by pathogen clearance. *PLoS One* **10**, e0125945.
- 509 **Jenkins TP, Formenti F, Castro C, Piubelli C, Perandin F, Buonfrate D, Otranto D, Griffin**
510 **JL, Krause L, Bisoffi Z and Cantacessi C** (2018b) A comprehensive analysis of the faecal
511 microbiome and metabolome of *Strongyloides stercoralis* infected volunteers from a non-
512 endemic area. *Scientific Reports* **8**, 15651.
- 513 **Jenkins TP, Peachey LE, Ajami NJ, MacDonald AS, Hsieh MH, Brindley PJ, Cantacessi C**
514 **and Rinaldi G** (2018a) *Schistosoma mansoni* infection is associated with quantitative and
515 qualitative modifications of the mammalian intestinal microbiota. *Scientific Reports* **8**, 12072.
- 516 **Jenkins TP, Rathnayaka Y, Perera PK, Peachey LE, Nolan MJ, Krause L, Rajakaruna RS**
517 **and Cantacessi C** (2017) Infections by human gastrointestinal helminths are associated with
518 changes in faecal microbiota diversity and composition. *PLoS One* **12**, e0184719.
- 519 **Kay GL, Millard A, Sergeant MJ, Midzi N, Gwisai R, Mduluza T, Ivens A, Nausch N,**
520 **Mutapi F and Pallen M** (2015) Differences in the faecal microbiome in *Schistosoma*
521 *haematobium* infected children vs. uninfected children. *PLoS Neglected Tropical Diseases* **9**,
522 e0003861.
- 523 **Kelly BJ, Gross R, Bittinger K, Sherrill-Mix S, Lewis JD, Collman RG, Bushman FD and**
524 **Li H** (2015) Power and sample-size estimation for microbiome studies using pairwise distances
525 and PERMANOVA. *Bioinformatics* **31**, 2461-2468.
- 526 **Kim JY, Kim EM, Yi MH, Lee J, Lee S, Hwang Y, Yong D, Sohn WM and Yong TS** (2018)
527 Intestinal fluke *Metagonimus yokogawai* infection increases probiotic *Lactobacillus* in mouse
528 cecum. *Experimental Parasitology* **193**, 45-50.
- 529 **Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, Gundra UM, Cho I, Bonneau R,**
530 **Blaser MJ, Chua KH and Loke P** (2014) Helminth colonization is associated with increased
531 diversity of the gut microbiota. *PLoS Neglected Tropical Diseases* **8**, e2880.

- 532 **Leung JM, Graham AL and Knowles SCL** (2018) Parasite-microbiota interactions with the
533 vertebrate gut: synthesis through an ecological lens. *Frontiers in Microbiology* **9**, 843.
- 534 **Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML,**
535 **Tucker TA, Schrenzel MD, Knight R and Gordon JI** (2008) Evolution of mammals and their
536 gut microbes. *Science* **320**, 1647-1651.
- 537 **Li RW, Li W, Sun J, Yu P, Baldwin RL and Urban JF** (2016) The effect of helminth infection
538 on the microbial composition and structure of the caprine abomasal microbiome. *Scientific*
539 *Reports* **6**, 20606.
- 540 **Lindgreen S, Adair KL and Gardner PP** (2016) An evaluation of the accuracy and speed of
541 metagenome analysis tools. *Scientific Reports* **6**, 19233.
- 542 **Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK and Knight R** (2012) Diversity,
543 stability and resilience of the human gut microbiota. *Nature* **489**, 220-230.
- 544 **Martin I, Djuardi Y, Sartono E, Rosa BA, Supali T, Mitreva M, Houwing-Duistermaat JJ**
545 **and Yazdanbakhsh M** (2018) Dynamic changes in human-gut microbiome in relation to a
546 placebo-controlled anthelmintic trial in Indonesia. *PLoS Neglected Tropical Diseases* **12**,
547 e0006620.
- 548 **Mutapi F** (2015) The gut microbiome in the helminth infected host. *Trends in Parasitology* **31**,
549 405-406.
- 550 [Matias Rodrigues JF, Schmidt TSB, Tackmann J and von Mering C \(2017\) MAPseq: highly](#)
551 [efficient k-mer search with confidence estimates, for rRNA sequence analysis. *Bioinformatics* **33**,](#)
552 [3808-3810.](#)
- 553 **O'Connell EM and Nutman TB** (2016) Molecular diagnostics for soil-transmitted
554 helminths. *The American Journal of Tropical Medicine and Hygiene* **95**, 508-513.
- 555 **Peachey LE, Jenkins TP and Cantacessi C** (2017) This gut ain't big enough for both of us. Or
556 is it? Helminth-microbiota interactions in veterinary species. *Trends in Parasitology* **33**, 619-632.

- 557 **Peachey LE, Molena RA, Jenkins TP, Di Cesare A, Traversa D, Hodgkinson JE and**
558 **Cantacessi C** (2018) The relationships between faecal egg counts and gut microbial composition
559 in UK thoroughbreds infected by cyathostomins. *International Journal for Parasitology* **48**, 403-
560 412.
- 561 **Pearce EJ and MacDonald AS** (2002). The immunobiology of schistosomiasis. *Nature Reviews*
562 *Immunology* **2**, 499-511.
- 563 **Pérez-Muñoz ME, Bergstrom K, Peng V, Schmaltz R, Jiménez-Cardona R, Marsteller N,**
564 **McGee S, Clavel T, Ley R, Fu J, Xia L and Peterson DA** (2014) Discordance between changes
565 in the gut microbiota and pathogenicity in a mouse model of spontaneous colitis. *Gut Microbes* **5**,
566 286-295.
- 567 [Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J and Glöckner FO](#)
568 [\(2013\) The SILVA ribosomal RNA gene database project: improved data processing and web-](#)
569 [based tools. *Nucleic Acids Research* **41**, 590-596.](#)
- 570 **Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, Honda K, Gause WC, Blaser**
571 **MJ, Bonneau RA, Lim YA, Loke P and Cadwell K** (2016) Helminth infection promotes
572 colonization resistance via type 2 immunity. *Science* **352**, 608-612.
- 573 **Rapin A and Harris NL** (2018) Helminth-bacterial interactions: cause and consequence. *Trends*
574 *in Immunology* **39**, 724-733.
- 575 [Rausch S, Held J, Fischer A, Heimesaat MM, Kühl AA, Bereswill S and Hartmann S](#) (2013)
576 [Small intestinal nematode infection of mice is associated with increased enterobacterial loads](#)
577 [alongside the intestinal tract. *PLoS One* **8**, e74026.](#)
- 578 **Reynolds LA, Smith KA, Filbey KJ, Harcus Y, Hewitson JP, Redpath SA, Valdez Y, Yebra**
579 **MJ, Finlay BB and Maizels RM** (2014) Commensal-pathogen interactions in the intestinal tract:
580 Lactobacilli promote infection with, and are promoted by, helminth parasites. *Gut Microbes* **5**,
581 522-532.
- 582 **Rosa BA, Supali T, Gankpala L, Djuardi Y, Sartono E, Zhou Y, Fischer K, Martin J, Tyagi**
583 **R, Bolay FK, Fischer PU, Yazdanbakhsh M and Mitreva M** (2018) Differential human gut
584 microbiome assemblages during soil-transmitted helminth infections in Indonesia and
585 Liberia. *Microbiome* **6**, 33-018-0416-5.
- 586 **Schneeberger PHH, Coulibaly JT, Gueuning M, Moser W, Coburn B, Frey JE and Keiser**
587 **J** (2018b) Off-target effects of tribendimidine, tribendimidine plus ivermectin, tribendimidine
588 plus oxantel-pamoate, and albendazole plus oxantel-pamoate on the human gut
589 microbiota. *International Journal for Parasitology Drugs and Drug Resistance* **8**, 372-378.

- 590 **Schneeberger PHH, Coulibaly JT, Panic G, Daubenberger C, Gueuning M, Frey JE and**
591 **Keiser J** (2018a) Investigations on the interplays between *Schistosoma mansoni*, praziquantel
592 and the gut microbiome. *Parasites & Vectors* **11**, 168-018-2739-2.
- 593 **Sekirov I, Russell SL, Antunes LC and Finlay BB** (2010) Gut microbiota in health and
594 disease. *Physiological Reviews* **90**, 859-904.
- 595 **Stensvold CR and van der Giezen M** (2018) Associations between gut microbiota and common
596 luminal intestinal parasites. *Trends in Parasitology* **34**, 369-377.
- 597 **Su C, Su L, Li Y, Long SR, Chang J, Zhang W, Walker WA, Xavier RJ, Cherayil BJ and**
598 **Shi HN** (2018) Helminth-induced alterations of the gut microbiota exacerbate bacterial
599 colitis. *Mucosal Immunology* **11**, 144-157.
- 600 **Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van-Hyleckama Vlieg JE, Strissel**
601 **K, Zhao L, Obin M and Shen J** (2015) Modulation of gut microbiota during probiotic-mediated
602 attenuation of metabolic syndrome in high fat diet-fed mice. *The ISME Journal* **9**, 1-15.
- 603 **Wang WL, Xu SY, Ren ZG, Tao L, Jiang JW and Zheng SS** (2015) Application of
604 metagenomics in the human gut microbiome. *World Journal of Gastroenterology* **21**, 803-814.
- 605 **World Health Organisation** (2006) Preventive chemotherapy in human helminthiasis:
606 coordinated use of anthelmintic drugs in control interventions: a manual for health professionals
607 and programme managers. Geneva: WHO; 2006.
- 608 **Wu S, Li RW, Li W, Beshah E, Dawson HD and Urban JF Jr.** (2012) Worm burden-dependent
609 disruption of the porcine colon microbiota by *Trichuris suis* infection. *PLoS One* **7**, e35470.
- 610 **Yatsunencko T, Rey FE, Manary MJ, Trehan I, Domínguez-Bello MG, Contreras M, Magris**
611 **M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J,**
612 **Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R and Gordon**
613 **JI** (2012) Human gut microbiome viewed across age and geography. *Nature* **486**, 222-227.
- 614
- 615

616 FIGURE LEGENDS

617 Fig. 1 Sources of variation and confounding factors potentially impacting the outcome of studies
618 of human-helminth-gut microbiota interactions in helminth-endemic regions.

619 Fig. 2 Proposed approaches aimed at reducing the methodological sources of variation
620 surrounding investigations of human-helminth-gut microbiota interactions.

For Peer Review