Cortés, A., Peachey, L., Scotti, R., Jenkins, T. P., & Cantacessi, C. (2019). Helminth-microbiota cross-talk: A journey through the vertebrate digestive system. *Molecular and Biochemical Parasitology*, *233*, [111222]. https://doi.org/10.1016/j.molbiopara.2019.111222

MOLBIO-2019-64.R1

Helminth-microbiota cross-talk – a journey through the vertebrate digestive system

Alba Cortés[1], Laura Peachey[1,2], Riccardo Scotti[1], Timothy P Jenkins[1] and Cinzia Cantacessi[1*]

[1]Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES, Cambridge, United Kingdom

[2] Bristol Veterinary School, Faculty of Health Sciences, University of Bristol, Langford House, Langford, BS40 5DU, Bristol, United Kingdom

Running title: Host-helminth-microbiota interactions in the vertebrate gut

*Corresponding author:
Cinzia Cantacessi
Department of Veterinary Medicine, University of Cambridge, Madingley Road CB3 0ES, Cambridge, United Kingdom
Tel. +44 (0) 1223 760541
Fax. +44 (0)1223 337610
cc779@cam.ac.uk

Abstract

The gastrointestinal (GI) tract of vertebrates is inhabited by a vast array of organisms, that is, the microbiota and macrobiota. The former is composed largely of commensal microorganisms, which play vital roles in host nutrition and maintenance of energy balance, in addition to supporting the development and function of the vertebrate immune system. By contrast, the macrobiota includes parasitic helminths, which are mostly considered detrimental to host health via a range of pathogenic effects that depend on parasite size, location in the GI tract, burden of infection, metabolic activity, and interactions with the host immune system. Sharing the same environment within the vertebrate host, the GI microbiota and parasitic helminths interact with each other, and the results of such interactions may impact, directly or indirectly, on host health and homeostasis. The complex relationships occurring between parasitic helminths and microbiota have long been neglected; however, recent studies point towards a role for these interactions in the overall pathophysiology of helminth disease, as well as in parasite-mediated suppression of inflammation. Whilst several discrepancies in qualitative and quantitative modifications in gut microbiota composition have been described based on host and helminth species under investigation, we argue that attention should be paid to the systems biology of the gut compartment under consideration, as variations in the abundances of the same population of bacteria inhabiting different niches of the GI tract may result in varying functional consequences for host physiology.

Key words

Helminth-microbiota interactions, gastrointestinal tract, bacterial 16S rRNA sequencing, microbial richness, microbial diversity, lactobacilli, *Prevotella*.

Table of contents

1. Worms and bacteria in the vertebrate gastrointestinal tract

The mammalian gastrointestinal (GI) tract is inhabited by trillions of microbes including bacteria, archaea, fungi, protozoa and viruses, that together form the gut microbiota (Table 1) [1,2]. This complex community of organisms plays essential roles in vertebrate physiology that include, for instance, nutrient metabolism, immune system development and protection against pathogens (cf. [3]). In many areas of the world, the GI tract is also home to other organisms, i.e. parasitic helminths, which are primarily pathogens [4]. Being co-habitants of the GI tract, the gut microbiota and parasitic helminths interact with each other, with likely consequences for host homeostasis and overall health (cf. [5]). For instance, significant disturbances in microbiota formation and stabilisation in early life due to high burdens of helminth infections (such as those occurring in endemic areas) may have far-reaching and long-term detrimental effects on host welfare [5,6]. On the other hand, the known immune-suppressive properties of GI helminths have been hypothesized to stem, at least in part, from direct and/or immune-mediated interactions between the parasites and the resident microbiota (cf. [7]).

Over the last few years, several studies have attempted to better understand the effect(s) that GI helminth infections exert on the composition and function of the vertebrate gut microbiota, with a view towards identifying key microbial players that could be exploited for the development of novel parasite control strategies and/or of helminth-based anti-inflammatory therapeutics (cf. [8,9]). Thus far, studies of helminth-microbiota interactions have been conducted in a range of host and parasite species of both public health and veterinary relevance, in substantially different geographical areas and experimental settings and with varying methodologies [8,10]. While published studies often acknowledge the intrinsic difficulties of performing comparative analyses of datasets generated from distinct host-parasite pairs, geographical locations, and/or with different experimental protocols, one aspect that is often overlooked is the specific site of the GI tract where helminth infections and/or associated changes in gut microbiota composition occur. Indeed, the functions of the resident microbiota (Table 1) vary according to the site of colonisation [3]; in addition, GI helminths are characterised by substantially different modes of interaction

with the gut mucosa (e.g. luminal *vs*. tissue-dwellers), as well as feeding strategies (e.g. grazers *vs*. blood-feeders) that may directly and/or indirectly shape their crosstalk with the resident bacteria. Studies of the systems biology of helminth-microbiota interactions must therefore consider these aspects. In this article, we review current knowledge of helminth-microbiota relationships according to infection site and parasite biology, and discuss potential functional differences and similarities between alterations in selected microbial populations following helminth colonisation of various GI compartments.

2. A hotchpotch of parasites, hosts and (micro)environments

In this review, and according to available published studies on helminth-gut microbiota crosstalk, the GI tract will be cursorily divided into stomach, small and large intestine. When available, details on GI sub-compartments (e.g. duodenum and colon within the small and large intestine, respectively) will be provided.

2.1. Stomach

To date, and to the best of our knowledge, studies that have characterised changes of gut microbial composition associated with parasitic helminths residing in the stomach of vertebrate hosts have involved nematodes of the abomasum of ruminants [11,12,13,14]. The abomasum is the fourth and last compartment of the ruminant stomachs, and is often referred to as the 'true stomach' due to its primary digestive function, that resembles that of monogastric animals. In the abomasum, the secretion of hydrochloric acid inactivates rumen microorganisms, while pepsins carry out the initial digestion of microbial and dietary proteins, which are further digested and absorbed in the small intestine [15,16]. Notwithstanding, the abomasum is still an important colonisation site for several microorganisms and acts as a barrier for bacterial transfer to the lower GI tract [17].

Abomasal infections by the trichostrongyle nematodes *Haemonchus contortus* and *Teladorsagia circumcincta* in small ruminants, and *Ostertagia ostertagi* in cattle, are characterised by alterations of the secretory activities of the stomach epithelium, and result in increased abomasal

pH (from ~2 to >6) and hyper-gastrinemia [18,19,20]. Early studies based on bacterial culture had reported that elevated abomasal pH is associated with the expansion of populations of anaerobic bacteria [18,20]. Thus, recently, high-throughput sequencing of the bacterial 16S rRNA gene (Table 1) has been applied to the characterisation of qualitative and quantitative changes of the composition of the microbiota colonising the abomasum of trichostrongyle-infected livestock, in order to better understand the potential metabolic and pathophysiological consequences of these alterations. In particular, a study conducted in partially immune cattle following reinfection with *O. ostertagi* reported negligible differences in abomasal microbiota structure prior to and following infection, and no significant changes in microbial alpha diversity (Table 1) nor in the relative abundances of any of the bacterial taxa identified [11]. This result led the authors to hypothesize that partially immune animals may develop the ability to maintain the stability of the abomasal microbial ecosystem, and thus of the gastric functions, in the presence of parasite infections [11].

Microbial alpha diversity was also unchanged in the abomasa of naïve goats following infection with *H. contortus* [12] and in faecal samples of lambs infected with *T. circumcincta* [14], which suggests that other yet unknown factors, independent of previous exposure to parasites and development of protective immunity, may participate in the interplay between helminths and bacteria residing in the vertebrate stomach. However, in spite of the lack of alpha diversity modifications in the abomasal microbiota of *H. contortus*- and *T. circumcincta*-infected goats and lambs, respectively, alterations in the relative abundances of several microbial taxa were detected in the stomachs of these animals [12,14]. In particular, several genera belonging to the family *Prevotellaceae*, including *Prevotella*, were expanded in the abomasa of infected goats [12], as well as in faecal samples of lambs infected with *T. circumcincta* [14] (Fig. 1). Members of the genus *Prevotella* participate in a range of metabolic functions in the rumen, including peptide degradation (e.g. [21,22]). Thus, the increased abundance of abomasal *Prevotella* that follows nematode infection has been hypothesised to represent a possible compensatory mechanism aimed to counteract infection-associated protein deficiency [12]. However, given that, in

ruminants, most of the microbiota-mediated proteo- and peptidolysis occurs in the rumen (i.e. the second gastric compartment; [15,16]), the role(s) of helminth-associated modifications in abomasal microbial flora in maintaining protein metabolism remain(s) unclear.

Intriguingly, the proliferation of *Prevotella* species in the gut has been associated with local (i.e., intestinal; [23,24,25,26]) and systemic [27,28] inflammation. Therefore, it seems plausible that, in addition to their roles in protein metabolism, expanded populations of *Prevotella* may contribute to the inflammation caused by the developing larvae [14]. Moreover, along with the expansion of *Prevotella* species, reduced populations of bacteria of the family *Lachnospiraceae*, and of the genus *Butyrivibrio* in particular, were detected in the abomasa of goats infected with *H. contortus* [12] (Fig. 1). These bacteria produce butyrate, a short-chain fatty acid (SCFA; see Table 1) with anti-inflammatory properties [3]. Thus, the reduction of these populations may exacerbate mucosal inflammation caused by helminth infections, a hypothesis that is yet to be tested.

2.2 Small intestine

The small intestine of vertebrates is the primary site of nutrient absorption and, due to its proximity to the stomach, it hosts a relatively small number of resident microorganisms [29]. Notwithstanding, the small intestinal flora participates in key biological pathways that include vitamin synthesis, lipid absorption and amino and bile acids metabolism, in addition to providing signals that regulate gut development and function [3].

A number of GI helminths of public health and veterinary importance inhabit the small intestine, including nematodes, trematodes and cestodes (e.g. [4]). Given the profound heterogeneity of these groups, studies investigating helminth-microbiota interactions involving small intestinal parasites will be considered according to their biology, i.e. as blood-feeders (hookworms), and tissue- and luminal-dwelling helminths, respectively (i.e. species characterised by an intra-intestinal tissue stage of development and species that establish in the gut lumen).

2.2.1. Blood-feeding helminths (hookworms). Hookworms, e.g. *Necator americanus* and *Ancylostoma duodenale*, are small blood-feeding nematodes (~1 cm in length) that, at the adult stage, reside in the small intestine of their vertebrate hosts; here, hookworms use 'teeth' or 'cutting plates' (depending on species) to attach to the mucosal lining and lacerate capillaries, causing small albeit chronic haemorrhages [30]. The effects that natural infections by *N. americanus* and/or *A. duodenale* exert on the composition of the gut microbiota of human hosts has been assessed mostly in individuals from geographical areas where soil-transmitted helminths (STHs) are endemic [31,32,33]. Thus, given that such infections commonly involve multiple parasite species, attributing observed modifications in gut microbial profiles to sole hookworm infections is unattainable [34]. Nevertheless, quantitative and qualitative changes in the composition of the human microbial flora in response to infections by *N. americanus* have been explored in studies in which hookworms were experimentally administered to a cohort of volunteers suffering from coeliac disease [35,36,37]. In these studies, hookworm infection was associated with an increase in gut microbial richness (Table 1), detected both in faecal and duodenal tissue samples, that was maintained following gluten challenge; such increase was hypothesised to contribute to the anti-inflammatory properties of these parasites *via* the restoration of microbial and immune homeostasis [36,37].

However, in order to gain a better understanding of the role(s) that parasite-associated changes in gut microbiota play in helminth-mediated immune-suppression, suitable experimental models are required (cf. [9]). A key study conducted in mice experimentally infected with the murine hookworm *Nippostrongylus brasiliensis* has provided experimental evidence of the role of the gut flora in helminth-driven immune-modulation [38]. In particular, the ileal microbiota of mice infected with *N. brasiliensis* was characterised by a significantly decreased beta diversity (Table 1), alongside expanded populations of *Lactobacillaceae*, and reduced *Turicibacteriaceae* and *Candidatus Arthromitus* (the latter belonging to the segmented filamentous bacteria = SFB) [38]. Notably, the contraction of SFB populations in the small intestine was dependent on the ability of the vertebrate host to initiate Th2-mediated immune responses against *N. brasiliensis* that

resulted in IL-13-driven changes in the architecture of the intestinal mucus and production of antimicrobial peptides, and were accompanied by significant downregulation of IL-17-encoding transcripts [38].

2.2.2. Tissue-dwelling helminths. Alterations in gut microbiota composition following infection with tissue-dwelling helminths of the small intestine have been best studied in mice experimentally infected with the duodenal nematode *Heligmosomoides polygyrus* [39,40,41,42]. Nevertheless, since ingested *H. polygyrus* larvae penetrate the submucosa of the small intestine of the rodent hosts and moult twice before emerging into the lumen as adult worms (cf. [43]), it must be pointed out that reported quantitative and qualitative changes in gut microbiota composition following *H. polygyrus* infection may be associated, at least partially, with the colonisation of the intestinal lumen by the adult stages of these parasites.

Investigations conducted in this host-parasite pair have provided key evidence of the interplay between helminth parasites, small intestinal microbiota and host immunity [41,42]. Whilst significantly expanded populations of *Lactobacillaceae* and/or the genus *Lactobacillus* have been consistently detected in the small intestine of susceptible C57BL/6 mice following infection by *H. polygyrus* [41,39,44], significant reductions in communities of *Lactobacillaceae* have been reported in partially resistant BALB/c mice (cf. [41,42]), which suggests the existence of a fine interplay between *H. polygyrus*, lactobacilli and host susceptibility to infection. Indeed, a causal relationship amongst these players was demonstrated by Reynolds and co-authors [41] in an elegant study showing positive correlations between intestinal loads of *Lactobacillaceae*, worm burdens and Treg frequencies within mesenteric lymph nodes (Fig. 1). In addition, in a separate study, the small intestinal microbiota of C57BL/6 mice infected with *H. polygyrus* was characterised by expanded populations of *Lachnospiraceae* (another family of SCFA-producing bacteria) and elevated SCFA levels [45] (Fig. 1). Intriguingly, *Lactobacillaceae* and *Lachnospiraceae* were also increased in the small intestinal microbiota of mice infected with the intracellular nematode *Trichinella spiralis* [46], while the genus *Lactobacillus* was expanded in

the same GI compartment of mice infected with *Strongyloides venezuelensis* [47]; however, whether these alterations participate in mechanisms of regulation of Treg responses in these models of infection is yet to be ascertained (Fig. 1).

Alongside studies conducted in murine models of parasite infection, investigations of the impact of helminth colonisation on the composition of the gut flora have also been conducted in naturally parasitized humans [48], as well as experimentally infected rabbits [49]. The former study explored the effects of long-term, subclinical monoparasitic infections by the nematode of the small bowel of humans, *Strongyloides stercoralis*, on resident bacterial populations [48]. *S. stercoralis* is characterised by a complex life cycle, that involves endogenous reinfections (= 'autoinfection') *via* filariform larvae that invade the mucosa of the large intestine, before emerging and establishing in the small intestine, where parthenogenetic adult females live threaded in the epithelium (reviewed by [50]). The faecal microbiota of chronically infected individuals from a non-endemic area of Europe was characterised by an increased microbial alpha diversity in comparison with that of uninfected controls from the same region [48]; this was accompanied by lower abundances of opportunistic and/or potentially pathogenic bacteria (e.g. *Bacteroides eggerthi* and *Pseudomonas* spp.), as well as increased proportions of bacteria within the class Clostridia [48]. Members of the latter class are known to contribute to the maintenance of gut homeostasis and modulation of immune tolerance *via* the production of SCFAs, amongst other mechanisms [51]. However, in this study, levels of SCFAs in the faecal metabolome of *Strongyloides*-infected individuals were comparable to those detected in samples from uninfected controls [48].

Other studies have suggested that host nutrition may play a key role in helminth-associated alterations in gut microbiota composition [49,52]. Amongst these, a recent investigation conducted in rabbits experimentally infected with *Trichostrongylus retortaeformis* highlighted the impact of diet in host-parasite-microbiota crosstalk [49]. This trichostrongyle colonizes the lumen of the duodenum, where adult parasites establish following a brief phase of larval development within the mucosa [53]. In coprophagic rabbits, *T. retortaeformis* infection was

accompanied by reduced gut microbial diversity and overall bacterial loads at the site of adult establishment [49], whilst restriction of coprophagy resulted in increased microbial diversity and bacterial community abundance in the duodenal mucosa of helminth-infected animals [49]. Furthermore, a number of compositional changes were associated with helminth infection irrespective of the nutritional input (i.e. coprophagy restriction/allowance), which could also impact on the metabolic capacity of the gut microbiota; for instance, the reported expansion of *Desulfocella* could contribute to fatty acid oxidizing functions in infected rabbits, whilst cellulolytic bacteria of the genera *Ruminoccocus* and *Bacteroides* were less abundant in infected animals compared to uninfected controls [49]. In addition, in the same study, changes in gut microbiota composition following infection were linked to the onset of host immune responses; in particular, expanded populations of *Lactobacillaceae* in the small intestine of *T. retortaeformis*-infected rabbits were positively correlated with levels of expression of T-bet, a key transcription factor defining Th1 cells [54], but not with those of the Treg-related molecules Foxp3 and IL-10 [49] (Fig. 1). This finding differs from the abovementioned commensal-pathogen-immunity interplay observed in mice experimentally infected with *H. polygyrus* [41]; however, it agrees with observations from two independent studies conducted in a murine model of chronic infection with the large intestinal nematode *Trichuris muris* ([55,56] – see section 2.3). Overall, this information adds weight to the hypothesis that, in spite of the similarities in bacterial taxa whose abundances are affected by helminth infections, the functional consequences of such changes may not be equal across host-parasite pairs.

In addition to the extensive research involving parasitic nematodes, recent studies have started to elucidate the impact that schistosome egg migration through the intestinal wall exerts on gut flora homeostasis [57,58]. Whilst *Schistosoma mansoni* and *S. japonicum* eggs penetrate the mucosa of both the small and large intestines [59], for the purpose of this review these parasites are considered helminths of the small intestine since in mice (the host species in which relevant investigations have been conducted) *S. mansoni* eggs egress preferentially *via* the Peyer's patches [60]. A key study conducted in *S. mansoni*-infected mice in which the gut microbiota had been

depleted by long-term exposure to broad-spectrum antibiotics showed significantly reduced faecal egg counts and decreased intestinal granuloma formation, thus supporting a role for the resident microbial populations in infection immunopathology [61]. In a more recent study, *Lactobacillaceae* were up-regulated in the small intestine of infected mice before the onset of *S. mansoni* egg laying, which led the authors to hypothesize that, similarly to *H. polygyrus* (cf. [40]), *S. mansoni* establishment could stimulate the early expansion of populations of bacteria with immune-regulatory functions [57] (Fig. 1). It must be however pointed out that pre-patent infections by *S. mansoni* in mice are dominated by a Th1-type phenotype [59] and that associations between populations of *Lactobacillaceae* and Th1-mediated immune responses have been reported in other experimental models of helminth infection [49,55,56]. Furthermore, the genus *Lactobacillus* was negatively associated with patent infections by *S. japonicum* in two mouse strains [58] and associations between *S. mansoni* infections and *Lactobacillaceae* abundance were no longer detected following the onset of egg laying [57] (Fig. 1).

During patent infections, both *S. mansoni* and *S. japonicum* colonisation were associated with significantly reduced alpha diversity and increased beta diversity of gut microbial populations, likely a result of the initiation of inflammatory responses against the egressing eggs [57,58]. Excretion of *S. mansoni* eggs through the Peyer's patches is known to cause an overall decrease in the lymphoid cellularity of this tissue, that includes IgA-producing B cells [60]. Given that diversified repertories of secretory IgA (sIgA) are pivotal for the maintenance of gut microbial community homeostasis, and that the Peyer's patches are the main inductive sites for GI sIgA responses (reviewed by [62]), it is conceivable that disturbances of patch structure and sIgA production due to egressing eggs could contribute to the observed shift in gut microbial composition towards a 'dysbiotic' phenotype (Table 1) (cf. [57,58]). Alongside changes in microbial diversity, *Schistosoma* spp. infection was also associated with expanded populations of putative pro-inflammatory bacteria (e.g. *Dorea* and *Bacteroides acidifaciens*) following the onset of egg-laying [57], an observation consistent with the reduced intestinal pathology detected in *S. mansoni* infected, gut microbiota-depleted mice [61].

Whilst the abovementioned studies have been conducted in rodent models of schistosome infections, the impact of hepatointestinal and urogenital schistosomiasis on the composition of the human gut flora has been investigated by examining faecal samples of children from a disease-endemic region [63]; in particular, infection-associated alterations of microbial profiles were found to be moderate in comparison with uninfected controls [63]. Notwithstanding, in some subjects, patent infections were associated with the expansion of bacteria within the phylum Proteobacteria (family *Enterobacteriaceae*), and the presence of overt clinical signs (e.g. blood in stool and splenomegaly, simple splenomegaly or vomiting within three hours following praziquantel treatment; [63]). The growth of *Enterobacteriaceae* is exacerbated by inflammatory microenvironments and, in turn, contributes to the worsening of inflammation and disease (cf. [64]). Together, data obtained from studies conducted in both humans and animal models of schistosomiasis point towards a profound impact of parasite infection on gut microbial composition and/or potential role of different pro-inflammatory bacteria in disease immunopathology [57,58,63]. Furthermore, whilst the pro-inflammatory profile of the schistosome-associated microbiota is often attributed to egg migration through the intestinal wall [57,58], it is well established that hepatic disorders have an enormous impact on gut microbiota structure and composition [65] and, therefore, the potential contribution of egg-related liver fibrosis to the gut microbial dysbiosis observed in schistosome-infected hosts should not be disregarded.

2.2.3. *Luminal-dwelling helminths*. A number of helminth species from several taxonomic groups establish in the lumen of the small intestine of the vertebrate hosts. Amongst these, ascarid larvae undergo intra- or extra-intestinal tissue migration before establishing as adult worms in the small intestine of their vertebrate host; this phase of parasite migration and development is likely to affect (directly and/or indirectly) the composition and function(s) of gut microbial communities; nevertheless, to the best of our knowledge, data on the impact of the migratory phases of ascarid infections on the host gut microbiota is still unavailable, whereas several studies have examined the effect(s) of patent infections by ascarid parasites on populations of gut microbial communities

of human and animal hosts. In particular, the effects of infection by the large roundworm *Ascaris lumbricoides* on the human gut microbiota have been assessed in combination with other STHs in endemic areas [31,32,33,34,66,67]. Nevertheless, alterations in gut flora composition have been characterised in pigs experimentally infected with *Ascaris suum* and cats naturally infected by a phylogenetically related nematode species, i.e. *Toxocara cati* [52,68]. Acute swine infection by *A. suum* was associated with a trend towards an increased alpha diversity and expanded populations of *Succinivibrio* and *Turicibacter* along with reductions in *Lactobacillus* [52] (Fig. 1). In addition, a decrease in the concentration of gut microbiota-derived SCFAs was observed in the proximal colon of infected pigs [52]. This result contrasts observations by Zaiss and co-authors [45], who reported increased levels of these mediators in faeces of pigs chronically infected with *A. suum*; nonetheless, this discrepancy could be related to differences between the acute and chronic phases of the infection (i.e. 14 *versus* 56 days post infection, respectively) [52].

Similar to *Ascaris* spp., adult *Toxocara* spp. attach to the intestinal mucosa of the definitive hosts, where they establish following somatic migration of larval stages that begins with the invasion of the small intestinal mucosa [69]. Patent infections with *T. cati* were associated with increased abundances of gut members of the order Lactobacillales (similarly to *H. polygyrus*- and *S. mansoni*-infected mice, and rabbits infected with *T. retortaeformis* [41,49,57]; Fig. 1), as well as of the family *Enterococcaceae* and the genera *Enterococcus* and *Dorea*, and reduced populations of several members of the class Gammaproteobacteria in faecal samples from these animals [68].

For trematodes, a single study has investigated the compositional gut microbial alterations of mice infected with the hypo-virulent food-borne trematode *Metagonimus yokogawai* [70]. No significant changes in caecal microbial alpha diversity were detected in infected mice compared to uninfected controls; however, the former were characterised by a reduced beta diversity [70], suggesting a ‘stabilising’ effect of the infection on the host gut microbiota. In addition, infection was accompanied by the expansion of several families within the phylum Firmicutes and members of the Bacteroidetes; in particular, *Lactobacillus* spp. were significantly more abundant in the gut microbiota of mice following infection [70] (Fig. 1), in accordance with the findings from

previous studies conducted using faecal samples of hamsters and mice (C57BL/6 strain) infected with the liver flukes *Opisthorchis viverrini* [71] and *Clonorchis sinensis* [72], respectively, as well as in several abovementioned murine models of GI helminth infection (cf. [41,46,48]).

Alterations in gut microbial profile composition have also been assessed in several studies conducted in rats experimentally infected with the cyclophilid cestode *Hymenolepis diminuta* [73,74,75,76]. This parasite anchors to the small intestinal mucosa *via* the four suckers located on its scolex, without causing major disturbances in the host gut (e.g. [4]). Considerable inconsistencies have emerged from these studies, both regarding the specific gut microbial taxa affected by infection, as well as the magnitude of the recorded changes [73,74,75,76]. In spite of these discrepancies, most of these investigations reported no significant alterations in gut microbial alpha diversity following *H. diminuta* infection of naïve rats. Nonetheless, a single study observed that, following experimental induction of colitis, *H. diminuta* infection was associated with a rapid restoration of gut microbial alpha diversity compared to uninfected rats [76]. This finding further supports the hypothesis that the anti-inflammatory properties of selected GI helminths may be linked, at least in part, to their ability to promote a shift towards a 'healthy' gut microbiota phenotype by stimulating an increase in microbial alpha diversity [35,36,37,77].

In addition, it has been suggested that *H. diminuta* colonisation may promote the stability of the gut microbiota in response to inflammatory stimuli, such as enteric bacterial infections [74]. This hypothesis was not supported by the findings of Pomajbíková et al. [76], who detected large disruptions in microbiota composition following the induction of colitis in animals infected by *H. diminuta*; however, given the substantial differences between the experimental protocols employed in these studies (e.g. the nature of the inflammatory stimuli, age of the animals at infection, and site and time of sampling [74,76]), direct comparisons are unwarranted.

2.3. Large intestine

The large intestine of vertebrates houses the largest number of microbial species along the GI tract (~10^{10}–10^{11} bacteria per gram of intestinal content in humans [29]); the vast majority of these species are strict anaerobes that perform a variety of key biological functions, including vitamin synthesis, amino acid metabolism and production of SCFAs [3,29]. Substantial differences in the anatomy of the large intestine exist amongst vertebrate species, which likely reflect the adaptation of this organ to the relative proportions of indigestible food in the host diet and may influence the diversity and composition of the microbial communities inhabiting this GI compartment across different hosts [78].

Thus far, studies of the interactions between the large intestinal microbial flora and parasitic helminths have focussed solely on tissue-dwelling species, and particularly on whipworms (*Trichuris* spp.), whose adults live partially embedded in the colonic and caecal mucosa, feeding on host tissues *via* a yet not fully understood mechanism [79]. A milestone study has explored the potential role(s) of *Trichuris*-induced changes in gut microbial profiles in parasite-mediated suppression of clinical signs of chronic idiopathic diarrhea (CID) in a primate model [77]. In particular, the mucosal microbiota of the colon of macaques with CID (a model of human inflammatory bowel disease) exposed to infection by *T. trichiura* was characterised by increased alpha diversity, as well as variations in the abundances of several bacterial phyla, including decreased Cyanobacteria alongside expanded Bacteroidetes and Tenericutes, which were accompanied by significant amelioration of diarrhoea and weight gain [77]. The authors hypothesized that reversion of intestinal inflammation linked to parasite-elicited Th2-mediated immune responses might have been responsible for significant changes in the mucosal environment which, in turn, might have limited the growth of *Cyanobacteria* and favoured that of *Tenericutes* [77].

The relationships between whipworm colonisation, host immunity and gut microbiota composition have been the focus of recent studies conducted in murine models of *T. muris* infection [55,56,80]. In particular, in two independent studies, chronic (low-dose) *T. muris* infection was associated with a significant decrease in faecal/colonic microbial alpha diversity,

alongside increased beta diversity and alterations of the abundances of several bacterial taxa [55,56]. However, in contrast to these findings, *T. muris* infection did not result in significant changes in caecal microbial alpha- and beta diversities in mice exposed to acute (high-dose) *T. muris* infection [80]. The infection dose is known to influence the relative resistance/susceptibility of mice to *T. muris* [81]. In particular, low *Trichuris* burdens promote Th1-mediated immune responses that lead to the establishment of chronic infections, whereas high doses of whipworm ova lead to the onset of Th2-dependent protective immunity that result in the expulsion of parasites [82]. Thus, it is tempting to speculate that alterations in the composition of the large intestinal microbiota may depend upon the type of host immune response mounted against the parasite and the subsequent alterations in the mucosal environment. This hypothesis is supported by the results of a study by Ramanan et al. [83], that demonstrated that the ability of infections by *T. muris* to suppress intestinal inflammation in a mouse model of Crohn's disease is dependent on Th2-mediated immune responses that result in significant reductions of populations of pro-inflammatory bacteria [83].

On the other hand, chronic *T. muris* infections were consistently associated with significant reductions in members of the phylum Bacteroidetes, together with expansion of the order/family Lactobacillales/*Lactobacillaceae* and the genus *Mucispirillum* in the gut microbiota of colonised mice [55,56]. A significant expansion in populations of *Lactobacillaceae* has been reported following infection of rodents, lagomorphs and felids with a range of nematodes and trematodes inhabiting the small intestine (see above). Notably, similar to findings from rodent models of *S. mansoni* [59] and *T. retortaeformis* infection [49], the expansion of Lactobacillales/*Lactobacillaceae* in the gut microbiota of *T. muris*-chronically infected mice occurred in an environment where Th1-mediated immune responses are dominant, whereas no correlation was reported between the relative abundances of these bacterial taxa and populations of Tregs [55,56] (Fig. 1). Potential roles of GI populations of *Lactobacillaceae* in type 1 inflammation are yet to be characterised; nevertheless, the data above are supported by

observations from Duque-Correa et al. [80], who detected expanded *Lactobacillaceae* populations following *T. muris* infection in mice lacking IL-10 signalling.

On the other hand, decreased numbers of Treg cells in the colonic lamina propria during chronic *T. muris* infection, corresponded to reduced abundances in populations of Bacteroidetes, and in particular of *Prevotella* and *Parabacteroides* [55,56]. Members of the Bacteroidetes, including *Prevotella*, play key roles in the anaerobic breakdown of dietary carbohydrates that result in the production of SCFAs [84] (Fig. 1). Consequently, it has been hypothesized that reduced populations of these bacterial taxa following *T. muris* infection could negatively impact on the microbial-dependent metabolism of the large intestine (whose microbial communities, particularly in hind gut fermenting omnivores and herbivores, play crucial roles in host nutrition *via* the metabolism of plant material) and the production of immunomodulatory SCFAs.

The colonic microbiota of pigs experimentally infected with *T. suis* was also characterised by significant changes in the relative abundances of several microbial taxa, that included reduced populations of *Ruminococcus*, *Succinivibrio* and *Oscillibacter*, and increased *Paraprevotellaceae* [85,86] and *Mucispirillum* [85]; the latter was consistent in mice chronically infected with *T. muris* [55,56] and, since this genus colonizes the mucus layer of the GI tract [87,88], their expansion has been attributed to the increased mucus production following infection [55,85]. This hypothesis is supported by observations by Duque-Correa et al. [80], who reported concomitant reduction of populations of *Mucispirillum* and loss of mucin-secreting goblet cells in IL-10 signalling-deficient mice infected by *T. muris*.

Moreover, in the gut microbiota of *T. suis*-infected pigs, the abundance of *Campylobacter* was significantly higher than that detected in the microbiota of uninfected controls, which raised the question of whether parasite infections could increase the susceptibility of pigs to colonisation by pathogenic *Campylobacter* [86]. A higher abundance of *Campylobacter* spp. has also been reported in the gut microbiota of horses with patent large intestinal helminth infections [89,90] (Fig. 1).

In equine species, the impact of large intestine-dwelling nematodes, i.e. the Cyathostominae, on gut microbiota composition has been recently investigated using faecal samples from chronically infected adult mares [91], as well as from foals with acute cyathostominosis [90]. Once ingested, cyathostomin larvae invade and encyst within the large intestinal mucosa of their equine hosts, to subsequently emerge in the gut lumen to complete their development to adult males and females [92]. Whilst a reduced gut microbial richness was associated with acute infection in young parasitized animals, a trend towards an increased gut microbial alpha diversity was observed in chronically infected adults [90,91]. These differences suggest that parasite-mediated alterations in gut microbiota composition in this host-parasite pair differ according to age-based alterations in immune responses mounted against these helminths, a hypothesis that requires thorough testing. Indeed, a separate study showed that anthelmintic treatment resulted in a rapid and transient decrease in faecal alpha diversity following cyathostomin removal in both yearlings and adult horses [93]. Comparative analyses of microbial populations whose abundances were altered in the presence of cyathostomin infection between adult and young animals suggest that compositional changes in gut microbiota composition could be also linked to the stage of infection [90,91]. In particular, chronic infections were associated with expanded populations of Elusimicrobia and Deltaproteobacteria, and reduced Methanomicrobia [91]. Conversely, acute infections were linked to expanded *Eubacteriaceae* and reduced *Lachnospiraceae*, both belonging to the class Clostridia [90]. Interestingly, reduced populations of *Prevotella* were also observed in the gut microbiota of infected yearlings [88], in accordance with previous observations in rodent models of *T. muris* infection [56], whilst reduced populations of *Lachnospiraceae* have been reported in the gut microbiota of horses susceptible to strongyle infection when compared to animals with natural resistance to these parasites [89] (Fig. 1). Notably, reductions in *Lachnospiraceae* communities were partially attributed a role in abomasal inflammation of goats infected with *H. contortus* [12] (Fig. 1).

3. Concluding remarks and future directions

The three-way interactions occurring between helminth parasites, the GI microbiota and the host gut are highly diverse in nature. On one hand, the vertebrate gut is a complex system that includes different compartments with specialised physiological functions, each populated by a highly diverse microbiota, in both composition and immune and metabolic activities [29]. As a result of such a diversity, the impact of GI helminth infections on populations of resident microbes largely depends on the host and parasite species under investigation [8].

In spite of such variation, some findings have been repeatedly observed in a number of hosts parasitized by helminths occupying different niches along the GI tract; amongst these, the expansion of populations of lactobacilli upon helminth colonisation is one the most frequently reported observations [41,46,47,49,55,56,57,70,80] (Fig. 1). Whilst the expansion of these bacteria was demonstrated to promote the establishment of *H. polygyrus* infections *via* the enhancement of Treg-mediated responses in mice [41], increased populations of *Lactobacillaceae*/*Lactobacillus* in other host-parasite systems was not associated with activation of regulatory immune responses [49,55,56,57,80] (Fig. 1). These contrasting observations suggest that the ability of lactobacilli to regulate host adaptive immune responses may be dependent on the activation of other immune-molecular pathways at the site of the infection that are yet to be elucidated. The frequently observed discrepancies between the 'gut microbial phenotype' resulting from helminth infections and the functional consequences of alterations in gut microbial composition highlight the need for thorough investigations aimed to identify the biological mechanisms regulating host-parasite-gut microbiota interactions, as well as their biological significance [9].

Furthermore, in studies of host-parasite-microbiota interactions, attention should be paid to the systems biology of the gut compartment under consideration (i.e. the interplay between the gut physiology, the resident microbiota and the mucosal immune system), as variations in the abundances of the same population of bacteria inhabiting different GI compartments may result in varying functional consequences for the host.

In addition to this, future studies should address the suitability of investigating faecal microbial composition as a proxy of bacterial populations inhabiting different compartments of the GI tract. Indeed, whilst consistent findings were reported for both stool samples and abomasal content from ruminants infected by abomasal nematodes [12,14], gut microbial alterations that followed infection by *H. diminuta* could not be consistently reproduced from caecal and faecal samples [2,49,80].

The establishment of a public repository compiling currently available bacterial 16S rRNA datasets from available studies of helminth-host-microbiota interactions may assist comparative analyses of the effects that a range of GI helminth infections exert on the microbial composition of different gut compartments and host species. Notwithstanding, in order to gain further insights into the biological significance of these observed alterations, sequencing data must be accompanied by additional investigations using other 'omics' technologies (e.g. transcriptomics, proteomics, metabolomics and immunomics), thus enabling a 'systems biology' view of the host-parasite-gut microbiome triad. In turn, this improved knowledge will represent a solid basis for translational applications aimed to discover and develop novel parasite control/treatment strategies and/or helminth-based anti-inflammatory therapeutics *via* the rational manipulation of the host gut microbiota.

References

1. Rajilic-Stojanovic M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev. 2014;38 5:996-1047.

2. Parfrey LW, Walters WA, Knight R. Microbial eukaryotes in the human microbiome: ecology, evolution, and future directions. Front Microbiol. 2011;2:153.

3. Brestoff JR, Artis D. Commensal bacteria at the interface of host metabolism and the immune system. Nat Immunol. 2013;14 7:676-684.

4. Levinson W. Review of medical microbiology and immunology (14th edn), McGraw-Hill Education. 2016.

5. Glendinning L, Nausch N, Free A, Taylor DW, Mutapi F. The microbiota and helminths: sharing the same niche in the human host. Parasitology. 2014;141 10:1255-1271.

6. Menzies SK, Rodriguez A, Chico M, Sandoval C, Broncano N, Guadalupe I, et al. Risk factors for soil-transmitted helminth infections during the first 3 years of life in the tropics; findings from a birth cohort. PLoS Negl Trop Dis. 2014;8 2:e2718.

7. Brosschot TP, Reynolds LA. The impact of a helminth-modified microbiome on host immunity. Mucosal Immunol. 2018; 11:1039-1046..

8. Peachey LE, Jenkins TP, Cantacessi C. This gut ain't big enough for both of us. or is it? Helminth-Microbiota Interactions in Veterinary Species. Trends Parasitol. 2017;33 8:619-632.

9. Cortes A, Toledo R, Cantacessi C. Classic models for new perspectives: delving into helminth-microbiota-immune system interactions. Trends Parasitol. 2018;34 8:640-654.

10. Rapin A, Harris NL. Helminth-bacterial interactions: cause and consequence. Trends Immunol. 2018;39 9:724-733.

11. Li RW, Wu S, Li W, Huang Y, Gasbarre LC. Metagenome plasticity of the bovine abomasal microbiota in immune animals in response to *Ostertagia ostertagi* infection. PLoS One. 2011;6 9:e24417.

12. Li RW, Li W, Sun J, Yu P, Baldwin RL, Urban JF. The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome. Sci Rep. 2016;6:20606.

13. El-Ashram S, Al Nasr I, Abouhajer F, El-Kemary M, Huang G, Dincel G, et al. Microbial community and ovine host response varies with early and late stages of *Haemonchus contortus* infection. Vet Res Commun. 2017;41 4:263-277.

14. Cortés A, Wills J. Su X, Hewitt R, Scotti R, Robertson J, et al. Infection with the gastrointestinal nematode *Teladorsagia circumcincta* increases tissue T cell numbers and luminal pathobionts. Submitted

15. Moran J. 5. How the rumen works. In Tropical dairy farming: feeding management for small holder dairy farmers in the humid tropics. Moran J (ed) Landlinks. 2005;42-49.

16. Hartinger T, Gresner N, Sudekum KH. Does intra-ruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation. J Anim Sci Biotechnol. 2018;9:33-018.

17. Lei Y, Zhang K, Guo M, Li G, Li C, Li B, et al. Exploring the spatial-temporal microbiota of compound stomachs in a pre-weaned goat model. Front Microbiol. 2018;9:1846.

18. Nicholls CD, Hayes PR, Lee DL. Physiological and microbiological changes in the abomasum of sheep infected with large doses of *Haemonchus contortus*. J Comp Pathol. 1987;97 3:299-308.

19. Purewal A, Fox MT, Shivalkar P, Carroll AP, Uche UE, Vaillant C, et al. Effects of *Ostertagia ostertagi* on gastrin gene expression and gastrin-related responses in the calf. J Physiol. 1997;498 3:809-816.

20. Simcock DC, Joblin KN, Scott I, Burgess DM, Rogers CW, Pomroy WE, et al. Hypergastrinaemia, abomasal bacterial population densities and pH in sheep infected with *Ostertagia circumcincta*. Int J Parasitol. 1999;29 7:1053-1063.

21. Walker ND, McEwan NR, Wallace RJ. A pepD-like peptidase from the ruminal bacterium, *Prevotella albensis*. FEMS Microbiol Lett. 2005;243 2:399-404.

22. Matsui H, Ogata K, Tajima K, Nakamura M, Nagamine T, Aminov RI, et al. Phenotypic characterization of polysaccharidases produced by four *Prevotella* type strains. Curr Microbiol. 2000;41 1:45-49.

23. Heimesaat MM, Bereswill S, Fischer A, Fuchs D, Struck D, Niebergall J, et al. Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*. J Immunol. 2006;177 12:8785-8795.

24. Lucke K, Miehlke S, Jacobs E, Schuppler M. Prevalence of *Bacteroides* and *Prevotella* spp. in ulcerative colitis. J Med Microbiol. 2006;55 Pt 5:617-624.

25. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011;145 5:745-757.

26. Dillon SM, Lee EJ, Kotter CV, Austin GL, Gianella S, Siewe B, et al. Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T-cell activation in untreated HIV-1 infection. Mucosal Immunol. 2016;9 1:24-37.

27. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202.

28. Maeda Y, Kurakawa T, Umemoto E, Motooka D, Ito Y, Gotoh K, et al. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. Arthritis Rheumatol. 2016;68 11:2646-2661.

29. Hillman ET, Lu H, Yao T, Nakatsu CH. Microbial ecology along the gastrointestinal tract. Microbes Environ. 2017;32 4:300-313.

30. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm infection. N Engl J Med. 2004;351 8:799-807.

31. Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, et al. Helminth colonization is associated with increased diversity of the gut microbiota. PLoS Negl Trop Dis. 2014;8 5:e2880.

32. Martin I, Djuardi Y, Sartono E, Rosa BA, Supali T, Mitreva M, et al. Dynamic changes in human-gut microbiome in relation to a placebo-controlled anthelminthic trial in Indonesia. PLoS Negl Trop Dis. 2018;12 8:e0006620.

33. Rosa BA, Supali T, Gankpala L, Djuardi Y, Sartono E, Zhou Y, et al. Differential human gut microbiome assemblages during soil-transmitted helminth infections in Indonesia and Liberia. Microbiome. 2018;6 1:33-018.

34. Cortés A, Peachey LE, Scotti R, Jenkins TP, Cantacessi C. Helminths and microbes within the vertebrate gut – not all studies are created equal. Parasitology. 2019, In press.

35. Cantacessi C, Giacomin P, Croese J, Zakrzewski M, Sotillo J, McCann L, et al. Impact of experimental hookworm infection on the human gut microbiota. J Infect Dis. 2014;210 9:1431-1434.

36. Giacomin P, Zakrzewski M, Jenkins TP, Su X, Al-Hallaf R, Croese J, et al. Changes in duodenal tissue-associated microbiota following hookworm infection and consecutive gluten challenges in humans with coeliac disease. Sci Rep. 2016;6:36797.

37. Giacomin P, Zakrzewski M, Croese J, Su X, Sotillo J, McCann L, et al. Experimental hookworm infection and escalating gluten challenges are associated with increased microbial richness in celiac subjects. Sci Rep. 2015;5:13797.

38. Fricke WF, Song Y, Wang AJ, Smith A, Grinchuk V, Mongodin E, et al. Type 2 immunity-dependent reduction of segmented filamentous bacteria in mice infected with the helminthic parasite *Nippostrongylus brasiliensis*. Microbiome. 2015;3:40-015.

39. Walk ST, Blum AM, Ewing SA, Weinstock JV, Young VB. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. Inflamm Bowel Dis. 2010;16 11:1841-1849; doi:10.1002/ibd.21299.

40. Rausch S, Midha A, Kuhring M, Affinass N, Radonic A, Kuhl AA, et al. Parasitic nematodes exert antimicrobial activity and benefit from microbiota-driven support for host immune regulation. Front Immunol. 2018;9:2282.

41. Reynolds LA, Smith KA, Filbey KJ, Harcus Y, Hewitson JP, Redpath SA, et al. Commensal-pathogen interactions in the intestinal tract: lactobacilli promote infection with, and are promoted by, helminth parasites. Gut Microbes. 2014;5 4:522-532.

42. Su C, Su L, Li Y, Long SR, Chang J, Zhang W, et al. Helminth-induced alterations of the gut microbiota exacerbate bacterial colitis. Mucosal Immunol. 2018;11 1:144-157.

43. Reynolds LA, Filbey KJ, Maizels RM. Immunity to the model intestinal helminth parasite *Heligmosomoides polygyrus*. Semin Immunopathol. 2012;34 6:829-846.

44. Rausch S, Held J, Fischer A, Heimesaat MM, Kuhl AA, Bereswill S, et al. Small intestinal nematode infection of mice is associated with increased enterobacterial loads alongside the intestinal tract. PLoS One. 2013;8 9:e74026.

45. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, et al. The Intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation. Immunity. 2015;43 5:998-1010.

46. Osborne LC, Monticelli LA, Nice TJ, Sutherland TE, Siracusa MC, Hepworth MR, et al. Coinfection. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation. Science. 2014;345 6196:578-582.

47. Pace F, Carvalho BM, Zanotto TM, Santos A, Guadagnini D, Silva KLC, et al. Helminth infection in mice improves insulin sensitivity via modulation of gut microbiota and fatty acid metabolism. Pharmacol Res. 2018;132:33-46.

48. Jenkins TP, Formenti F, Castro C, Piubelli C, Perandin F, Buonfrate D, et al. A comprehensive analysis of the faecal microbiome and metabolome of *Strongyloides stercoralis* infected volunteers from a non-endemic area. Sci Rep. 2018;8 1:15651-018.

49. Cattadori IM, Sebastian A, Hao H, Katani R, Albert I, Eilertson KE, et al. Impact of helminth infections and nutritional constraints on the small intestine microbiota. PLoS One. 2016;11 7:e0159770.

50. Viney M. *Strongyloides*. Parasitology. 2017;144 3:259-262.

51. Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog. 2013;5 1:23-4749.

52. Williams AR, Krych L, Fauzan Ahmad H, Nejsum P, Skovgaard K, Nielsen DS, et al. A polyphenol-enriched diet and *Ascaris suum* infection modulate mucosal immune responses and gut microbiota composition in pigs. PLoS One. 2017;12 10:e0186546.

53. Audebert F, Vuong PN, Durette-Desset MC. Intestinal migrations of *Trichostrongylus retortaeformis* (Trichostrongylina, Trichostrongylidae) in the rabbit. Vet Parasitol. 2003;112 1-2:131-146.

54. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol. 2013;13 11:777-789.

55. Holm JB, Sorobetea D, Kiilerich P, Ramayo-Caldas Y, Estelle J, Ma T, et al. Chronic *Trichuris muris* infection decreases diversity of the intestinal microbiota and concomitantly increases the abundance of lactobacilli. PLoS One. 2015;10 5:e0125495.

56. Houlden A, Hayes KS, Bancroft AJ, Worthington JJ, Wang P, Grencis RK, et al. Chronic *Trichuris muris* infection in C57BL/6 mice causes significant changes in host microbiota and metabolome: effects reversed by pathogen clearance. PLoS One. 2015;10 5:e0125945.

57. Jenkins TP, Peachey LE, Ajami NJ, MacDonald AS, Hsieh MH, Brindley PJ, et al. *Schistosoma mansoni* infection is associated with quantitative and qualitative modifications of the mammalian intestinal microbiota. Sci Rep. 2018;8 1:12072-018.

58. Zhao Y, Yang S, Li B, Li W, Wang J, Chen Z, et al. Alterations of the mice gut microbiome via *Schistosoma japonicum* ova-induced granuloma. Front Microbiol. 2019;10:352.

59. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. Nat Rev Immunol. 2002;2 7:499-511.

60. Turner JD, Narang P, Coles MC, Mountford AP. Blood flukes exploit Peyer's Patch lymphoid tissue to facilitate transmission from the mammalian host. PLoS Pathog. 2012;8 12:e1003063.

61. Holzscheiter M, Layland LE, Loffredo-Verde E, Mair K, Vogelmann R, Langer R, et al. Lack of host gut microbiota alters immune responses and intestinal granuloma formation during schistosomiasis. Clin Exp Immunol. 2014;175 2:246-257.

62. Lycke NY, Bemark M. The role of Peyer's patches in synchronizing gut IgA responses. Front Immunol. 2012;3:329.

63. Schneeberger PHH, Coulibaly JT, Panic G, Daubenberger C, Gueuning M, Frey JE, et al. Investigations on the interplays between *Schistosoma mansoni*, praziquantel and the gut microbiome. Parasit Vectors. 2018;11 1:168-018.

64. Zeng MY, Inohara N, Nunez G. Mechanisms of inflammation-driven bacterial dysbiosis in the gut. Mucosal Immunol. 2017;10 1:18-26.

65. Wahlstrom A. Outside the liver box: The gut microbiota as pivotal modulator of liver diseases. Biochim Biophys Acta Mol Basis Dis. 2019;1865 5:912-919.

66. Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M, et al. Patent human infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in the faecal microbiota. PLoS One. 2013;8 10:e76573; doi:10.1371/journal.pone.0076573.

67. Jenkins TP, Rathnayaka Y, Perera PK, Peachey LE, Nolan MJ, Krause L, et al. Infections by human gastrointestinal helminths are associated with changes in faecal microbiota diversity and composition. PLoS One. 2017;12 9:e0184719.

68. Duarte AM, Jenkins TP, Latrofa MS, Giannelli A, Papadopoulos E, de Carvalho LM, et al. Helminth infections and gut microbiota - a feline perspective. Parasit Vectors. 2016;9 1:625-016.

69. Sprent JF. The life history and development of *Toxocara cati* (Schrank 1788) in the domestic cat. Parasitology. 1956;46 1-2:54-78.

70. Kim JY, Kim EM, Yi MH, Lee J, Lee S, Hwang Y, et al. Intestinal fluke *Metagonimus yokogawai* infection increases probiotic *Lactobacillus* in mouse cecum. Exp Parasitol. 2018;193:45-50.

71. Plieskatt JL, Deenonpoe R, Mulvenna JP, Krause L, Sripa B, Bethony JM, et al. Infection with the carcinogenic liver fluke *Opisthorchis viverrini* modifies intestinal and biliary microbiome. FASEB J. 2013;27 11:4572-4584.

72. Kim JY, Kim EM, Yi MH, Lee J, Lee S, Hwang Y, et al. Chinese liver fluke *Clonorchis sinensis* infection changes the gut microbiome and increases probiotic *Lactobacillus* in mice. Parasitol Res. 2019;118 2:693-699.

73. McKenney EA, Williamson L, Yoder AD, Rawls JF, Bilbo SD, Parker W. Alteration of the rat cecal microbiome during colonization with the helminth *Hymenolepis diminuta*. Gut Microbes. 2015;6 3:182-193.

74. Williamson LL, McKenney EA, Holzknecht ZE, Belliveau C, Rawls JF, Poulton S, et al. Got worms? Perinatal exposure to helminths prevents persistent immune sensitization and cognitive dysfunction induced by early-life infection. Brain Behav Immun. 2016;51:14-28.

75. Wegener Parfrey L, Jirku M, Sima R, Jalovecka M, Sak B, Grigore K, et al. A benign helminth alters the host immune system and the gut microbiota in a rat model system. PLoS One. 2017;12 8:e0182205.

76. Jirku Pomajbikova K, Jirku M, Leva J, Sobotkova K, Morien E, Parfrey LW. The benign helminth *Hymenolepis diminuta* ameliorates chemically induced colitis in a rat model system. Parasitology. 2018;145 10:1324-1335.

77. Broadhurst MJ, Ardeshir A, Kanwar B, Mirpuri J, Gundra UM, Leung JM, et al. Therapeutic helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory signature and mucosal microbiota of the colon. PLoS Pathog. 2012;8 11:e1003000.

78. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research?. Dis Model Mech. 2015;8 1:1-16.

79. Hansen TV, Hansen M, Nejsum P, Mejer H, Denwood M, Thamsborg SM. Glucose absorption by the bacillary band of *Trichuris muris*. PLoS Negl Trop Dis. 2016;10 9:e0004971.

80. Duque-Correa MA, Karp NA, McCarthy C, Forman S, Goulding D, Sankaranarayanan G, et al. Exclusive dependence of IL-10R alpha signaling on intestinal microbiota homeostasis and control of whipworm infection. PLoS Pathog. 2019;15 1:e1007265.

81. Cliffe LJ, Grencis RK. The *Trichuris muris* system: a paradigm of resistance and susceptibility to intestinal nematode infection. Adv Parasitol. 2004;57:255-307.

82. Bancroft AJ, Else KJ, Humphreys NE, Grencis RK. The effect of challenge and trickle *Trichuris muris* infections on the polarisation of the immune response. Int J Parasitol. 2001;31 14:1627-1637.

83. Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, et al. Helminth infection promotes colonization resistance via type 2 immunity. Science. 2016;352 6285:608-612; doi:10.1126/science.aaf3229.

84. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54 9:2325-2340.

85. Li RW, Wu S, Li W, Navarro K, Couch RD, Hill D, et al. Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. Infect Immun. 2012;80 6:2150-2157.

86. Wu S, Li RW, Li W, Beshah E, Dawson HD, Urban JF. Worm burden-dependent disruption of the porcine colon microbiota by *Trichuris suis* infection. PLoS One. 2012;7 4:e35470.

87. Robertson BR, O'Rourke JL, Neilan BA, Vandamme P, On SL, Fox JG, et al. *Mucispirillum schaedleri* gen. nov., sp. nov., a spiral-shaped bacterium colonizing the mucus layer of the gastrointestinal tract of laboratory rodents. Int J Syst Evol Microbiol. 2005;55 Pt 3:1199-1204.

88. Berry D, Schwab C, Milinovich G, Reichert J, Ben Mahfoudh K, Decker T, et al. Phylotype-level 16S rRNA analysis reveals new bacterial indicators of health state in acute murine colitis. ISME J. 2012;6 11:2091-2106.

89. Clark A, Salle G, Ballan V, Reigner F, Meynadier A, Cortet J, et al. Strongyle infection and gut microbiota: profiling of resistant and susceptible horses over a grazing season. Front Physiol. 2018;9:272.

90. Peachey LE, Castro C, Molena RA, Jenkins TP, Griffin JL, Cantacessi C. Dysbiosis associated with acute helminth infections in herbivorous youngstock – observations and implications. Sci Rep. 2019;9:11121..

91. Peachey LE, Molena RA, Jenkins TP, Di Cesare A, Traversa D, Hodgkinson JE, et al. The relationships between faecal egg counts and gut microbial composition in UK thoroughbreds infected by cyathostomins. Int J Parasitol. 2018;48 6:403-412.

92. Corning S. Equine cyathostomins: a review of biology, clinical significance and therapy. Parasit Vectors. 2009;2 Suppl 2:S1-3305.

93. Walshe N, Duggan V, Cabrera-Rubio R, Crispie F, Cotter P, Feehan O, et al. Removal of adult cyathostomins alters faecal microbiota and promotes an inflammatory phenotype in horses. Int J Parasitol. 2019;49 6:489-500.

LEGENDS TO FIGURES

Fig. 1. Populations of gut bacteria reportedly affected by gastrointestinal helminth infections. Double-entry chart summarising the alterations in the abundances of populations of selected members of the vertebrate gut microbiota (rows) that have been repeatedly reported following infection with gastrointestinal helminths, listed according to the gastrointestinal compartment where parasite infection occurs (columns). For each pair of bacteria/infection site, the reported change in bacterial population abundance (increase/decrease; arrows), host (icon) and parasite pair, and site of sampling (in brackets) are indicated. The proposed effects on host metabolism and/or immunity are summarised in the right column (grey). NR = no changes reported.

1