This gut ain't big enough for both of us. Or is it? Helminth-microbiota interactions in veterinary species

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Gastrointestinal helminth parasites share their habitat with a myriad of other organisms, i.e. the commensal microbial flora. Increasing evidence, particularly in humans and rodent models of helminth infection, points towards a multitude of interactions occurring between parasites and the gut microbiota, with a profound impact on both host immunity and metabolic potential. Despite this information, the exploration of the effects that parasite infections exert on the commensal gut microbes of veterinary species is a field of research in its infancy. In this article, we summarise studies that have contributed to current knowledge of helminth-microbiota interactions in species of veterinary interest, and identify possible avenues for future research in this area, which could include the exploitation of such relationships to improve parasite control and delay or prevent the development of anthelmintic resistance.

Gut micro- and macrobiota: cooperation or competition?

The gastrointestinal (GI) tract of vertebrates is inhabited by a vast array of organisms, i.e. the **micro-** and **macrobiota** (see Glossary). The former is composed largely of commensal microorganisms, which play a vital role in host nutrition and maintenance of energy balance, in addition to supporting the development and function of the vertebrate immune system [1-3]. On the other hand, 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 [4]. Sharing the same environment within the vertebrate host, it is plausible that 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 [5-7]. For instance, helminths and microbiota compete for host nutrients while, in parallel, the known immune-modulatory properties of a range of parasites may translate into modifications of mucosal and systemic immunity to the resident bacteria [8]. The complex relationships occurring between helminths and microbiota have long been neglected; however, recent studies pointing towards a role of these interactions in the overall pathophysiology of helminth disease [5-7, 9-28] are drawing attention to this little-known area of research. Nevertheless, current knowledge of helminth-microbiota interplay relies heavily on studies of helminth-infected humans or rodent models [5, 7, 11-15, 18-22, 25, 26], while the impact that parasites exert on the commensal flora of species of veterinary interest is still poorly understood. Given the production losses and the considerable morbidity and mortality associated to a range of helminth diseases in livestock [29-33], as well as the global threat of emerging anthelmintic resistance [34-36], the exploration of the complexities of host-helminth-microbiota interactions in species of veterinary interest is timely and relevant. The implications of this newly acquired knowledge will be multiple, from a better understanding of the systems biology of parasites, to the collection of information that could form a solid basis for the development of novel intervention strategies against GI helminths. In this article we provide an overview of current knowledge of helminth-microbiota interactions in species of veterinary interest, suggest potential applications of this knowledge in veterinary clinical medicine, and outline avenues of future research that, in our view, will be pivotal to translate research findings into practice. Given that mice are, in principle, a veterinary species and that mouse models of infection are often used in veterinary research [37-39], data available for these hosts will be considered here alongside that from other animal species.

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A matter of (animal and helminth) species

Current studies of helminth-microbiota interactions in veterinary species involve a range of animals and parasites, and are characterised by a vast heterogeneity in experimental designs and techniques which, taken together, lead to a variety of findings (Table 1). In spite of these variations, a small number of specific changes in the composition of the host gut microbiota have been consistently observed in helminth-infected animals, irrespective of (host and parasite) species. Such changes are therefore likely to represent genuine helminth-associated alterations to the resident commensal flora. For instance, populations of *Lactobacillaceae*, gram positive bacteria of the phylum Firmicutes with an important role in carbohydrate metabolism [40], are frequently expanded in the presence of helminths in the GI tract of animals, including mice infected with the roundworm *Heligmosomoides polygyrus* [5, 6, 12, 14], the whipworm *Trichuris muris* [15] and the hookworm *Nippostrongylus braziliensis* [13]. Interestingly, *Lactobacillaceae* were also increased in the biliary ducts of hamsters infected by the trematode *Opisthorchis viverrini* [26], and in the faecal microbiota of cats with patent infections by the roundworm *Toxocara cati* [17]. *Lactobacillaceae* are known to exert immune-modulatory functions in the host gut, primarily by promoting an expansion of T regulatory cells, which underpins their use as a probiotic supplement for GI inflammatory diseases [41]. In particular, in a recent key study, Reynolds and co-workers [5] not only demonstrated that experimental infections of mice with *H. polygyrus* were accompanied by a marked expansion in populations of *Lactobacillaceae*, but also that increased worm burdens could be observed following administration of *Lactobacillus* species to mice prior to experimental parasite infection [5]. This finding led the authors to hypothesise the occurrence of a form of mutualism between *Lactobacillaceae* and selected GI helminths, whereby each promotes the activation of T regulatory mechanisms, thus reducing the effect of the host immune response on the counterpart. Unlike for the *Lactobacillaceae*, knowledge of the impact of GI helminth infections on populations of other microbes is inconsistent, being largely dependent on species of hosts and parasites under consideration. For instance, *Enterobacteriaceae* are increased in *H. polygyrus*-infected mice [5, 14]. As these bacteria are able to tolerate oxidative stress [42, 43], their expansion is linked to the onset of intestinal inflammation following parasite infection. In addition, a marked increase in bacteria of the genus *Mucispirillum* (family *Deferribacteraceae*) has been associated to infections by *T. muris* and *T. suis* in mice and pigs, respectively, likely as a consequence of the increased production of host mucin in response to helminth colonisation [11, 15, 24]. Conversely, the

microbiota of *T. muris* infected-mice displays a marked reduction in abundance of genera of the phylum Bacteroidetes, e.g. *Prevotella* and *Parabacteroides* [11, 15], which results in an overall decrease in microbial species **richness** and diversity (i.e. **alpha diversity**) in the GI tract.

The impact of helminth infections on microbial richness and diversity

Alpha diversity is defined as the mean species diversity within a population of microbes, and it is dependent on both **microbial richness** (i.e. the number of species making up a microbial population) and **evenness** (i.e. the relative abundance of each microbial species in a population) [44]. While an increased alpha diversity in the GI microbiota is generally associated with a 'healthy' gut homeostasis, many inflammatory GI and/or systemic diseases are accompanied by a reduced alpha diversity [45-47]. Consistent with this knowledge, a few studies have reported a marked decrease in alpha diversity in correspondence of the acute phase of infection by parasitic helminths. Examples include rabbits infected by the nematode *Trichostrongylus retortaeformis* [7] and mice infected by *T. muris* [11, 15]. In contrast, in humans and primates, natural or experimental infections by GI helminths (e.g. *T. trichiura* and *Necator americanus*) were accompanied by a general increase in microbial alpha diversity [18-20, 27]. However, in most studies conducted to date in a range of animal-helminth systems, the alpha diversity of the gut microbiota remained unchanged following parasite infection [6, 13, 16, 17, 23, 25, 28]. Whilst obvious differences in animal and parasite species, as well as in experimental set-ups, might account for these contrasting observations, it is plausible that the acute onset of inflammation that follows parasite invasion of the GI tract is accompanied by an initial decrease in microbial alpha diversity, and that this is restored (or increased) in concomitance with the establishment of chronic infections. Thus, the time of sampling, and hence the stage of parasite infection, is an important variable that may significantly impact on the findings of such studies. Nonetheless, determining the impact that helminths exert on the alpha diversity of the gut microbiota of species of veterinary interest, and particularly on that of livestock, is of paramount importance, as the gut metabolism of these species (and consequently their productivity) is greatly dependent on the maintenance of a 'healthy' commensal flora.

Helminth-associated alterations in host metabolism

Several studies have examined the functional effects of helminth infection on host metabolism [6, 7, 10, 11, 16, 23, 24], either directly by evaluating differences in levels of

148   faecal metabolites in infected *vs* non-infected hosts [11, 24], or indirectly by inferring

149   helminth-associated changes in host metabolism based on expansion or reduction of selected

150   bacterial populations in response to parasite infection [48]. Of note, bacterial taxa and/or

151   metabolic markers associated with fibrolytic potential and carbohydrate and protein transport

152   and metabolism have been shown to be altered in response to parasite infection [6, 10, 11, 16,

153   24]. In particular, studies in both *T. suis*-infected pigs and *T. muris*-infected mice have

154   inferred a down-regulation in these metabolic pathways in the colon [10, 11, 24]. In mice

155   infected by *T. muris,* suggested changes were linked to a reduction in *Prevotella* and

156   *Parabacteroides* (phylum Bacteroidetes), which are known to play an important role in

157   degradation of proteins and carbohydrates [49]. In contrast, increases in carbohydrate, protein

158   and lipid metabolism have been speculated to occur as a consequence *H. polygyrus* and

159   *Haemonchus contortus* infections in mice colons and goat abomasa, respectively [6, 16]; in

160   particular, in the latter study, such increases were concurrent with an expansion in *Prevotella*

161   species [16]. The authors of this study hypothesized that, given that infections by *H.*

162   *contortus* are generally associated with overall protein loss, changes in abomasal microbiota

163   in response to *Haemonchus*-driven pathology could reflect an attempt of the vertebrate host

164   to functionally compensate for protein deficiency [16]. Whether changes in microbiota

165   composition and metabolism are caused by direct interactions of the microbial flora with

166   helminth parasites or, indirectly, by changes in mucosal immunity as a response to parasite

167   infection, remains to be determined. Establishing causal relationships between helminths and

168   gut microbiota is nonetheless pivotal, as this knowledge will form the necessary basis for the

169   development of novel parasite control strategies based on the manipulation of the host

170   commensal flora.

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173   **Which came first, the chicken or the egg?**

174   Three main hypotheses have been formulated on the causality of relationships between

175   parasitic helminths and the resident commensal flora. In particular, helminth-associated

176   changes in gut microbiota could be (i) secondary to the host immune response to infection [5,

177   7, 13, 15], (ii) driven by the vertebrate host in a bid to create a hostile environment for the

178   parasite [50, 51] and (iii) the result of direct interactions with parasite excretory/secretory

179   (ES) products [14, 52] (Figure 1). The first hypothesis is supported by the findings of several

180   studies which correlate up-regulation of cytokines following parasite invasion with changes

181   in microbial composition [5, 7, 14, 15]. For instance, Cattadori and co-workers [7]

182 demonstrated that up-regulation of interferon (IFN) γ following infection of rabbits with *T.*
183 *retortaeformis* was associated with the expansion of *Pasteurellaceae, Clostridiaceae,*
184 *Ruminococcaceae, Peptostreptococcaceae* and *Flammenovirgaceae*, and that that the
185 *Enterobacteriaceae* were reduced in correspondence with up-regulation of Th2 cytokines [7].
186 Further support for this hypothesis was provided by a study by Fricke and colleagues [13],
187 who demonstrated that the effects of *N. braziliensis* infection on the composition of the
188 murine gut microbiota, host antimicrobial proteins (AMP) and IL-17 expression, were
189 attenuated in STAT6 -/- and IL-13 -/- knockout mice, thus presenting evidence of a role of
190 Th2 responses in parasite-associated modifications in the commensal flora [13]. However,
191 contrary to these findings, a study examining the effect of *H. polygyrus* on the composition of
192 the gut microbiota of laboratory mice recorded no differences in parasite-associated microbial
193 changes between IL4-α -/- knockout and wild type mice, thus indicating that, at least in this
194 instance, Th2 responses were not responsible for the observed modifications [14]. On the
195 other hand, evidence for an active role of the host in inducing changes in the gut microbiota
196 following helminth infection has been provided by observations that successful host
197 responses to helminth infection are linked to increased production of AMPs, such as
198 lysozymes in cattle [51] and angiogenin 4 in mice [50], albeit it was suggested that these
199 responses may represent a downstream effect of Th2-mediated immunity [13]. Finally,
200 although there is no direct evidence of a direct interaction between parasite ES and gut
201 microbiota, the ES products of *H. polygyrus* are known to contain lysozymes, which could
202 plausibly have a direct effect on GI microbiota [52]. From this set of observations, it is
203 evident that the causal relationships between infections by parasitic helminths and changes in
204 the composition of the commensal flora remain to be thoroughly investigated. While each of
205 the theories described above is unequivocally valid, the reality may be represented more
206 accurately by a complex community ecology scenario, whereby all of the factors described
207 above are inextricably linked. In the immediate future, dissecting these relationships will be
208 crucial, as knowledge of this area will enable host-parasite systems to be manipulated for
209 clinical benefit.

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212 **Potential avenues in veterinary research**
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214 Knowledge of helminth-microbiota interactions in veterinary species is advancing, and while
215 further work is required to improve our basic understanding in this field, the potential
216 possibilities to manipulate such interactions to the benefit of the vertebrate hosts are already

217   evident. For instance, *ad hoc* modifications of the host microbiota could be exploited to either
218   strengthen the host immune response against the parasite, artificially create a hostile
219   environment for the latter or minimise the negative effects of parasitism on host metabolism.
220   Indeed, the administration of a **probiotic** supplement [53] containing selected species of
221   *Lactobacillaceae* such as *L. taiwanensis,* and *L. casei,* is known to promote the establishment
222   of *H. polygyrus* in mice, *via* a reduction in Th2 cytokines such as IL-4 and IL-13 and an
223   increase in T regulatory CD4+ cells (see above) [5, 54]. This raises the question of whether
224   other microbial species might promote host immunity against parasite infection. Indeed, in
225   protozoal infections, e.g. by *Giardia intestinalis* and *Eimeria acervulina,* the administration
226   of probiotic bacteria (including members of the genera *Lactobacillus*, *Bifidobacterium*,
227   *Enterococcus*, *Pediococcus* and *Bacillus*) have been shown to promote host immune
228   responses [55-60] that, in the case of *Eimeria*, were driven by an expansion of mucosal
229   intraepithelial lymphocyte populations and a concomitant increase in the serum levels of
230   specific antibodies [57]. In addition, previous studies have recorded a marked reduction in the
231   intestinal stages of *Trichinella spiralis* in experimentally infected mice following
232   intraperitoneal or oral administration of *L. casei* [61-65]; in one instance, these observations
233   were accompanied by an increase in IL-4 and reduction in IFNγ [64], thus suggesting that the
234   administration of probiotics had promoted an effective Th2 response. Similarly,
235   administration of the probiotic *Bifidobacterium animalis* to mice prior to experimental
236   infections with *Strongyloides venezuelensis* has resulted in a significant reduction of worm
237   burdens [66]. This finding corroborated previous observations that expansions of
238   *Bifidobacterium* in humans and pigs are associated with lower burdens of helminth parasites
239   [20, 67]. Future studies should further explore the potential use of *Bifidobacterium* and other
240   probiotics to improve host response to helminth infections in veterinary species (Figure 2).
241   Unlike probiotics, **prebiotics** are dietary supplements composed of non-digestible plant
242   fibres, which promote the growth of resident gut microbes [68]. Prebiotics have been shown
243   to have profound effects on the outcome of helminth infections. A primary example comes
244   from the dietary supplementation of inulin in pigs [69-71, 72]. Inulin is a glycosidic fructan,
245   that is resistant to digestion in the small intestine of monogastric species, thus acting as a
246   bacterial substrate in the large intestine, particularly for Lactobacillales [73].
247   Supplementation of 16% dietary inulin results in 87% and 71% reductions in burdens of
248   *Oesophagostomum dentatum* and *T. suis*, respectively, in infected swine [69-71, 72]. High
249   levels of the products of **bacterial metabolism** of inulin, i.e. lactic acid and short chain fatty
250   acids, are thought to be responsible for this effect, as they lead to a reduction of the luminal

pH in the caecum and colon which, in turn, results in death and expulsion of adult worms [70] (Figure 2). However, thus far, no knowledge is available on the effect of inulin administration on the composition of the gut microbiota, which would greatly assist the identification of the bacterial populations implicated in the anthelmintic properties of inulin. In another study in pigs, diet supplementation with the natural forage chicory, that contains high levels of fructan, resulted in a 64% reduction in *Ascaris suum* burdens, which was associated with expanded populations of *Lachnospiraceae* and *Bifidobacterium* and reduced *Enterobacter* [67]. Interestingly, the same study reported increased *T. suis* burdens following the supplementation, which contrasts previous observations of the effects of inulin administration in this animal species [71, 72]. These discrepancies may be linked to differences in relative doses of the supplements, or to inherent differences between changes in the composition of gut microbiota associated to the administration of inulin and chicory, respectively. This contrasting information further emphasises the need for a concurrent evaluation of the effects of supplement administration to the composition of the commensal flora which, in our opinion, is a necessary step towards the evaluation of the promise of dietary interventions as a parasite control strategy alternative to the use of anthelmintics in veterinary species.

In addition to administering dietary supplements with anthelmintic properties, it is also plausible that dietary alterations *per se* could be exploited to improve host resilience and/or resistance to infection (Figure 2). Indeed, previous studies have indicated that helminth-associated alterations in GI microbiota in mice, pigs and rabbits may be linked to changes in the ability of the commensal flora to metabolise proteins, carbohydrates and lipids which, in turn, could result in production losses [6, 10, 11, 16, 24]. Interestingly, preventing the natural behaviour of coprophagy in rabbits infected with *T. retortaeformis* resulted in the restoration of prior helminth-associated perturbations in GI microbiota [7], thus indicating that some of the effects of parasitism on the host microbiota and metabolism could potentially be mitigated by **diet manipulation**. This data indicates that further, more comprehensive, investigations are needed in order to evaluate the real impact of helminth infections on the metabolic functions of the microbiota, and thus to develop strategies to minimise such effects and prevent helminth-associated production losses. Given the global threat of anthelmintic resistance worldwide, strategic manipulation of diet, in combination with good management practices, could represent the future of parasite control in production animals in a post-anthelmintics era.

Final considerations and future directions

The exploration of the mechanisms that govern the interactions between parasitic helminths and the gut microbiota in veterinary species has a number of implications for translational research in this field. Overall, thus far, research in this area is characterised mostly by inconsistent findings, with a few exceptions. The reasons for this are three-fold; firstly, observed changes in gut microbiota are likely to be unique to each host-helminth system, thus making comparisons between findings unwarranted. Secondly, the current literature is characterised by a heterogeneity of experimental designs, which span, beside host and helminth species, time and location of sampling and techniques used to characterise changes in the microbiota (Table 1 and Box 1). Indeed, all these variables are likely to have a profound impact on the changes observed and the repeatability of the experiments [6, 15]. In addition, the lack of appropriate negative control samples in a large number of studies published to date is likely to have led to misinterpretations of findings. Thirdly, subtle differences in the baseline composition of the microbiota and individual immune responses to helminth infections may heavily influence the outcomes of experiments, even in instances where the host-helminth system, sample location and time point, and analytical techniques are identical [12]. This knowledge highlights the need for repeatability before conclusions are drawn. Indeed, it is only through repeated observations of specific sets of findings that common 'truths' begin to emerge. In addition, where possible, a 'standardisation' of study designs will be crucial to minimise biases and, in our opinion, should involve sampling both the luminal and mucosally associated microbiota throughout the gut, and at several time-points corresponding to acute and chronic helminth infection. Importantly, in the future, investigations of the intimate mechanisms that govern the interplay between parasites and GI flora should include, besides the commensal bacteria, viruses and eukaryotes inhabiting the gut. Studies of helminth-microbiota interactions under natural conditions of (co)infections will also assist in translating laboratory findings to 'real life' clinical scenarios. Indeed, whilst knowledge to date suggests that the manipulation of the gut microbiota has the potential to make both war and peace with helminth infections in veterinary species, more studies are needed in order to make the most of this potentially powerful tool (see Outstanding Questions).

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501 **Glossary**

502

503 **Alpha diversity:** In ecology, the mean species diversity at the local, within-site or
504 within-habitat scale. It is dependent on both the number of species making up a
505 population (richness) and the relative abundance of each species in a population
506 (evenness).

507

508 **Diet manipulation:** A targeted feeding approach that is aimed at inducing a specific
509 physiological effect.

510

511 **Macrobiota:** The macroscopic flora and fauna of a region.

512

513 **Microbiota:** The microscopic flora and fauna of a region.

514

515 **Microbial evenness:** Microbial species similarity in abundance within an
516 environment or population.

517

518 **Microbial metabolism:** The chemical processes that occur within a microbe in order
519 to maintain life.

520

521 **Microbial richness:** Number of microbial species present in a given sample.

522

523 **Prebiotic:** Dietary supplements that allow specific changes in the composition and/or
524 activity in the gastrointestinal microflora.

525

526 **Probiotic:** Live micro-organisms which, when administered in adequate amounts,
527 confer a health benefit to the host.

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Box 1: Techniques for profiling of microbial populations

A range of techniques are available for microbial population profiling, each with *pros* and *cons* relating to data generation and analysis, and costs (Figure I). Amongst 'traditional' methods, culturing allows the identification and analysis of specific, 'target' bacteria; however a large number of microbial species inhabiting the vertebrate gut (>30%) are currently uncultivable [74]. Fluorescence in situ hybridization (FISH) uses fluorescently labelled oligonucleotide probes that are hybridised to complementary target bacterial 16S rRNA sequences, thus allowing separation of species through flow cytometry and subsequent phylogenetic identification [75]. Terminal restriction fragment length polymorphism (T-RFLP) consists in applying fluorescently labelled primers to amplify bacterial DNA, followed by digestion of the 16S rRNA amplicon through restriction enzymes, and separation by gel electrophoresis [76]. Conversely, in denaturing gradient gel electrophoresis/temperature gradient gel electrophoresis (DGGE/TGGE) the 16S rRNA amplicons are denatured by a denaturant/or temperature gradient within the gel, thus allowing for separation of bacterial taxa according to differences between sequences. Other techniques that allow both identification of bacterial taxa and semi-quantitation of taxon abundance include Sanger sequencing or qPCR of cloned bacterial 16S rRNA amplicons and DNA microarrays [77-79]. While cloning and qPCR target specific microbial groups, microarrays can be used for unbiased analyses of bacterial populations and overcome potential errors introduced by PCR amplification. More recently, studies of helminth-microbiota interactions have taken advantage of the availability of next generation sequencing technologies; these allow the unbiased evaluation of microbial populations while simultaneously providing data on relative abundance of individual species within each sample. These techniques can either rely on high-throughput amplification of the bacterial 16S rRNA gene (which includes a PCR step) or on the direct sequencing of whole bacterial genomes, as well as those of viruses and eukaryotic organisms, within each sample [80]. These techniques require specific expertise and are relatively costly.

566

567 **Table 1.** A summary of currently available studies on host-helminth-microbiota interactions in veterinary species, including study design,
568 microbiota profiling techniques and principal findings.

Host Species	Parasite species*	Time of sampling (days post infection)	Site (S)/type (T) of sample	Method of profiling microbiota	Effect on diversity	Predominant changes reported	Ref.
Rodents							
Mouse (*Mus musculus*) strain C57BL/6	*Trichuris muris* (N)	13, 20, 27, 35	S - caecum T - faeces, lumen	-High throughput sequencing of 16S rRNA amplicons (Illumina)	↓alpha diversity	Phylum:↑Firmicutes,↑Proteobacteria,↓Bacteroidetes Family:↑*Lactobacillaceae* Genus: ↑*Lactobacillus,* ↑*Mucispirillum* (caecum only)	[15]
Mouse (*Mus musculus*) strain C57BL/6	*Trichuris muris* (N)	14, 28, 42, 49, 56, 63, 70, 77, 84, 91	T – faeces	-Denaturing gradient gel electrophoresis - High throughput sequencing of 16S rRNA amplicons (454)	↓alpha diversity	Phylum:↓ Bacteroidetes Genus:↓*Prevotella*,↓*Parabacteroides*, ↑*Mucospirillium*	[11]
Mouse (*Mus musculus*) strain C57BL/6 wildtype and IL4α-/-	*Heligmosoides polygyrus* (N)	6, 14, 28	S – ileum, caecum, colon T – lumen	-Culture -Cloned 16S rRNA amplicon qPCR - Denaturing gradient gel electrophoresis	Not assessed	Class:↑ γ-Proteobacteria caecum Family: ↑*Enterobacteriaceae* caecum Genus:↑*Lactobacillus* ileum, ↑*Bacteroides* caecum	[14]
Mouse (*Mus musculus*) strain C57BL/6 and BALB	*Heligmosoides polygyrus* (N)	28	S –duodenum T – lumen, faeces	-qPCR	Not assessed	Family: ↑*Enterobacteriaceae*, ↑*Lactobacillaceae* (duodenum/faeces)	[5]
Mouse (*Mus musculus*) strain C57BL/6 (x2)	*Heligmosoides polygyrus* (N)	14	S – ileum, caecum T – mucosa	-Cloned 16S rRNA gene sequencing -qPCR total bacteria	Not assessed	Family: ↑*Lactobacillaceae* ileum	[12]
Mouse (*Mus musculus*) strain C57BL/6 wildtype and STAT6 -/- IL13-/-	*Nippostrongylus brasiliensis* (N)	11	S – small intestine T – lumen, faeces	-qPCR - High throughput sequencing of 16S rRNA amplicons (Illumina)	No change	Phylum: ↓Firmicutes ,↑Bacteroides,↑Actinobacteria Family:↑*Lactobacillaceae*, ↑S4-27 family (bacteroides), ↑*Coriobacteriaceae* Species: ↓*Candidatus arthromitus*	[13]
Wild mice (*Apodemus flavicollis*)	*Heligmosoides polygyrus* *Syphacia* spp. *Hymenolepsis* spp. (N, C)	N/A	S – stomach, ileum, caecum, colon T – lumen, mucosa	- High throughput sequencing of 16S rRNA amplicons (454)	No change (but no controls)	Phylum: *H. polygyrus* - ↓Bacteroides, ↑Firmicutes *Sypacia* spp.- ↑Bacteroides, ↓Firmicutes Family: *Hymenolepsis* spp ↑S4-27 (Bacteroides) stomach *H. polygyrus* ↑*Lactobacillaceae* ileum	[6]
Rat *(Rattus norvegicus)*	*Hymenolepsis diminuta* (C)	58	S – caecum T – lumen	- High throughput sequencing of 16S rRNA amplicons (Illumina)	No change	Family:↑*Peptostreptococcaceae* Genus:↓*Turibacter*	[25]

Host Species	Parasite species	Time of sampling (days post infection)	Site (S)/type (T) of sample	Method of profiling microbiota	Effect on diversity	Predominant changes reported	Ref.
Rodents							
Hamster (*Mesocricetus auratus*)	*Opisthorchis viverrini* (T)	42	S – bile ducts, colorectum T – lumen	- High throughput sequencing of 16S rRNA amplicons (454)	↑alpha diversity	Phylum: ↑Spirochaetes Family: ↑*Lachnospiraceae*, ↑*Ruminococcaceae*, ↑*Lactobacillaceae*, ↓*Porphyromonadaceae*, ↓*Erysipelotrichaceae*, ↓*Eubacteriaceae*	[26]
Rabbits (*Oryctolagus cuniculus*)	*Trichostrongylus retortaeformis* (N)	0,15,30,60	S – duodenum T – mucosa	- High throughput sequencing of 16S rRNA amplicons (454)	↓alpha diversity	Phylum:↑Proteobacteria, ↑Spirochaetes, ↓Firmicutes Family:↑*Leptospiraceae*, ↑*Desulfobacteraceae*, ↓*Ruminococcaceae*, ↓*Phyromonadaceae*, ↓*Bacteroidaceae* Genus: ↑*Leptomena*, ↑*Desulfocella*, ↓*Bacteroides* ↓*Ruminococcus*	[7]
Swine							
Pig (*Sus scrofa domestica*)	*Trichuris suis* (N)	53	S – colon T – lumen	-Whole metagenome shotgun sequencing (Illumina)	Not assessed	Pylum: ↓ Fibrobacteres, ↓ Spirochaetes, ↓Tenericutes, ↓Gammatimonadetes Genus:↓*Fibrobacter*, ↓*Treponema*, ↓*Dorea*, ↓*Ruminococcus*, ↑*Campylobacter*	[10]
Pig (*Sus scrofa domestica*)	*Trichuris suis* (N)	21	S – colon T – lumen	-Whole metagenome shotgun sequencing (454) -High throughput sequencing of 16S rRNA amplicons (454)	Not assessed	Pylum: ↑Deferribacteres, Proteobacteria? Genus:↓*Oscillobacter*, ↓*Succinivibrio*, ↑*Mucispirillum*, ↑*Paraprevotella*, ↑*Desulfovibrio*	[24]
Ruminants							
Goats (*Capra aegagrus hircus*)	*Haemonchus contortus* (N)	50	S – abomasum T – lumen	-High throughput sequencing of 16S rRNA amplicons (Illumina)	No change	Phylum: ↓Euryarchaeota Order:↑Pasteurellales Species:↑*Selenomonas ruminantium*	[16]
Cattle (*Bos taurus*)	*Ostertagia ostertagi* (N)	14	S – abomasum T – lumen	-High throughput sequencing of 16S rRNA amplicons (454)	No change	Genus: ↑*Ethanoligenens*, ↓*Subdoligranulum*	[23]
Companion animals							
Cats (*Felis catus*)	*Toxocara cati* (N)	One time point case control	T – faeces	-High throughput sequencing of 16S rRNA amplicons (Illumina)	No change	Phylum:↑Actinobacteria Class: ↑Coreobacteriia, ↓Gammaproteobacteria Order: ↑ Lactobacillales, ↑Coribacteriales Family: ↑*Enterococcaceae*, ↑*Coreobacteriaceae* Genera: ↑*Collinsella*, ↑*Enterococcus*, ↑*Dorea*, ↑*Lactobacillus*, ↑*Ruminococcus*, ↓ *Bulleidia*, ↓*Jeotgalicoccus*	[17]

Host Species	Parasite species	Time of sampling (days post infection)	Site (S)/type (T) of sample	Method of profiling microbiota	Effect on diversity	Predominant changes reported	Ref.
Companion animals							
Cats and dogs *(Felis catus* and *Canis lupus familiaris)*	*Ancylostoma caninum* (N) (co-infection with *Giardia* spp.)	One time point case control	T – faeces	-High throughput sequencing of 16S rRNA amplicons (bacterial tag encoded FLX amplicon pyrosequencing)	No change	No compositional changes due to *Ancylostoma caninum* alone in this study	[28]

569   * N = nematode, T = trematode, C = cestode

570   **Legends to figures**

571

572   **Figure 1. Current theories of causality of helminth-microbiota interactions in the**

573   **gastrointestinal system of vertebrate hosts.** (1) Helminth infections induce local

574   and systemic host immune responses which, in turn, impact on the composition of the

575   microbial flora; (2) the host epithelial cells produce antimicrobial proteins (AMP) in

576   response to helminth infections, with subsequent alteration of the microbial flora; (3)

577   Helminth excretory/secretory products (ES) induce shifts in the gut microbiota

578   composition.

579

580   **Figure 2. Potential use of microbiota manipulation for controlling helminth**

581   **infection and disease.** (A) Selected probiotics, e.g. *Bifidobacteria*, could be

582   administered to promote host Th2 immune responses leading to death and expulsion

583   of parasites; (B) Prebiotics, e.g. inulin, could be administered to promote growth of

584   selected bacterial taxa, e.g. lactobacilli, and increase in their metabolites (e.g. short

585   chain fatty acids (SCFAs) or lactic acids (LA)), leading to a decrease in gut pH and

586   helminth death and expulsion; (C) Diet manipulation, e.g. increased protein or

587   carbohydrate, could be used to counteract the changes in microbiota metabolism

588   associated to helminth infection.

589

590   **Figure I. Pros and cons of 'traditional' and 'modern' microbiota profiling**

591   **techniques.**

592
593