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Helminth Infections and Gut Microbiota: The Futuristic Study of Pathogen Virulence and Gut Ecosystem

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Abstract

The animal gut has co-evolved with the microbes and parasites leading to an increased tolerance to their existence therein. Their peaceful co-existence has been a subject of research in the recent years to decipher their probabilities in multidimensional applications ranging from eradicating the helminths without affecting gut homeostasis or using helminths as therapeutics. Negligence in any one of the components of the animal gut can lead to unhealthy gut ecosystems often manifesting a diseased state. Supply of key nutrients, synthesis of vitamins, resistance to invasion by pathogens and helping in hatching of helminth eggs are some important services provided by the gut bacteria that may well be utilised by the helminth. The gut bacteria thus seem to be exploited by the helminths for establishment of infection in the host. However, relationships are not as simple as it seems. The paper tries to present different aspects of this host-bacteria-helminth relationship and their future perspectives.

Keywords: Animal gut; Helminth bacteria; Co-existence; Co-evolution

Introduction

The animal gut apart from performing its normal functions of digestion and absorption provides shelter to the microbes and parasites, broadening the concept of the gut. The environment of the intestine can be considered as a complex ecosystem involving biological and chemical interactions among the host, parasites and microbiota that conditions the host gut [1]. While most of the research has been concentrated on the parasitic disease caused by the helminth itself, including the plethora of clinical symptoms and chemotherapeutic killing of the helminth (either the adult or larval forms) as the ultimate panacea; developing insights into the hows and whys of the helminth infection is shaping up currently. The co-existence of the helminth in the human (or animal) gut despite an active immune system is paradoxical in itself, immune down-regulation by helminths being the only acceptable answer so far. The concept of the "Superorganism" by Nicholson et al. [2] or the expansion of "Hygiene Hypothesis" [3] by Yazdanbakhsh and Matricardi [4] to include the helminth parasites or the "macrofauna" of the gut along with the microbial communities to condition the maturing immune system in becoming tolerant to the environmental antigens, have directed the researcher's thoughts towards more important and unseen ecological interactions in the animal gut. The gastrointestinal helminths and the microbial community of the gut have co-evolved together with the immune system of the host [5]. Understanding this process of co-evolution and the underlying evolutionary forces becomes an important key to the understanding of the co-existence of the micro and macrofauna in the animal gut. Though the relationships are still not clear [5], it does indicate some kind of interactive metabolism and modification of the molecules in the gut by each of the resident organism therein [6]. Study of these intertwined complicated relationship becomes a priority as it is important to know how the situations would be if any one of these components were removed. The interactive relationships are also important for developing new trends of treatment for helminth and/or bacterial infections as in case of treatment of IBD (Inflammatory Bowel Disease) with helminths [5].

The current paper discusses on the advances in this field of research and also tries to look into its future perspectives both as a diagonistic tool and a therapeutic tool.

Effects of the Presence of Helminths on Gut-Microbiota

Shifts in the habitat of the gut microbiota

It has been hypothesized that the secretory and excretory molecules produced by the gut helminths may in some way modify the host gut for the microbiota [6]. Studies by Walk et al. [7] revealed that *Heligmosomoides polygyrus* infections in mice have significant impact on the bacterial communities of the ileum but not the colon of the mice gut. Though *H. polygyrus* itself is a resident of the duodenum, the load of Lactobacillae species were found to be increased in the ileum and the end of the caecum [7]. Adult worms removed from the mice during necropsy were also found to be associated with these bacteria [7]. The 'traffic jam' concept given by Biswal et al. [8] also highlights how the unfavourable site (for microbial growth) in a bird's gut is altered to more convenient site (for microbial growth) simply by the presence of helminths in the gut. The intestine stuffed with helminths creates regions of relative stasis and the food cannot pass readily as it would in their absence [8]. This increases the bacterial density in the gut with helminths, as food is retained for a longer time allowing the bacteria to act on it [8]. Kreisinger et al. [9] also gave valuable inputs in this field by demonstrating alteration of the host gut microbiota depending on the species of helminth present in the gut. The study also revealed that the helminths could regulate the microbial communities both upstream and downstream of their location using *H. polygyrus*, Syphacia spp. and Hymenolepis spp in their experiments [9]. The results throw light to the potentiality of the helminths to alter the gut homeostasis [9]. The ability of the helmnith to regulate the gut microbial population, not necessarily directly on their location and/or depending on the species of helminth in question is very interesting since different species of helminths (alone

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or in combination) may be used to study such microbial regulation from the therapeutic viewpoint both for the humans and the veterinary animals.

Changes in Bacterial Population

Wu et al. [10] had studied the dynamics of the proximal colon microbiota in pigs in response to infection by *Trichuris suis*. The study reported an increase in populations of the pathogenic bacteria, Campylobacter spp. [11] in pigs affected with *T. suis*. Many species of bacteria like Oscillibacter and Succinivibrio were significantly decreased while Paraprevotella and Mucispirillum were increased in helminth infected pigs [10]. De Fillipo et al. [12] and Yatsunenko et al [13] have reported significant differences in the faecal microbial communities from the residents of developing countries in comparison to those from the developed countries. Reports of De Filippo et al. [12] show that members of the phylum, Prevotella were dominant in the faecal microbial populations of children from developing countries as compared to that of Europe and USA. A difference in the helminth infection status from these two places has been postulated as the probable reason for such variations [5]. In yet another study Li et al. [14] reported that *Ostertagia ostertagi* infection in cattle causes minimal changes to the microbial flora. It was hypothesized that they inhabit the abomasum where the pH is very low and probably the microbiota of this region was less sensitive to the immune responses generated by these helminths [5]. Infections by digenetic trematodes like *Schistosoma mansoni* have also been reported to cause alterations in the gut microbiota which was manifested by the presence of high levels of trimethylamine, phenylacetylglycine and ρ-cresol glucuronide in the urine of infected mice [15]. Similar changes were also detected in infections by *Fasciola hepatica*, *Necator americanus* and other human helminth parasites [16]. Balog et al. [17] had reported alterations in the gut microbial communities concomitant with *S. mansoni* on the basis of urinary response of rodent and human hosts. Li et al. [14] could differentiate between infected and uninfected individuals with respect to Schistosoma infection by presence of twelve urinary and five faecal metabolites as biomarkers in infected individuals thereby supporting the hypothesis that *S. mansoni* alters the host gut microbial activity. Leung and Loke [5] opined that immune responses of the intestinal mucosa in helminth infections seem to exert their effects on the modification of the gut microbiota. Lee et al. [18] studied the faecal samples from non-infected and helminth infected individuals and found an increase in members of Paraprevotellaceae family of bacteria in individuals with *Trichuris trichiura* infection. Similar comparative study on the faecal samples of celiac patients before and after giving a low dose of *Necator americanus* infection revealed increased bacterial populations in faeces of helminth infected individuals [19]. This part is extremely important as it opens a gateway for innovative biomarkers for diagnosing the health status. Humans are going to benefit from this undoubtedly but its applicability for other animals is encouraging as well. The metagenomic DNA analyses of the faecal samples of the zoo animals or the operational taxonomic unit (OTU) based analysis from the RNA database can give valuable information regarding the helminth status of these animals. The information may be used for the better conservation of zoo animals and the wild animals in a broader sense.

Role of the Gut Microbiota in Establishment of the Helminth Infection

Lowering the inflammatory responses

Comparative study of the helminth population (with respect to *H. polygyrus*) between conventional mice (with gut bacteria) and germfree mice (gut freed of bacteria) showed that the worm burden was higher in conventional mice as the germfree mice flushed out the worms from their gut quite rapidly [7]. Moreover, the intestinal mucosa in the germfree mice developed a robust eosinophilia which was not found in the conventional mice suggesting differential immune responses in the presence and absence of the gut bacteria [7]. Similar eosinophilia, granuloma and thickening of the small intestine in germfree mice were also reported much earlier by Wescott [20] based on experiments using *Nematospiroides dubius* (now known as *Heligmosomoides polygyrus*). The Th2 mediated immune responses generated by the helminths have been reported to play important roles in the expulsion of helminths and also protect the host tissue from severe damage [21]. However, the bypassing action of the helminth to establish chronic infections evading the host immune responses indicates a helminth-generated immunoregulatory network that reduces the inflammatory damage to both the parasite and the host [22]. Acute helminth infections have been associated with visible alterations in the gut microbiota [10]. Walk et al. [7] suggested that increased Lactobacillaceae family members may have important anti-inflammatory or immune suppressive effects in *H. polygyrus* infection. The Lactobacillaceae group has been reported to produce reactive oxygen species that inhibits the activation of transcription factor NF-κB in intestines of neonatal mouse [23], reverse intestinal injury [24] and provides protection against graft induced host disease [25]. Based on these properties of the Lactobacillaceae group, Glendinning et al. [26] hypothesized the existence of a symbiotic relationship between the helminths and the microbes to be one of the key players in reducing intestinal inflammation. Matsumoto et al. [27] has also documented the anti-inflammatory properties of some members of the genus *Lactobacillus*. The exact effects on the gut microbiota as a result of this cascade of reaction have not been investigated in details [5]. However, in some way the gut microbiota is a part of this reaction series and may be essential for the establishment of helminth infection by a subdued immune reaction in the intestine of the host. Studies in this field are still wanting.

Studies so far have been mostly based on direct modulation of the host immune system by the intestinal helminths. However, the findings of Zaiss et al. [28] gave a new dimension to this field of study. They demonstrated that the helminths enhance the production of short chain fatty acids (SCFAs) by the intestinal bacteria using the murine- *Heligmosomoides polygyrus* bakeri model. The SCFAs in turn promote host regulatory T cell responses and increased production of anti-inflammatory cytokines ultimately dampening the immune-pathological reactions [28]. These immune-suppressive properties of the bacteria-derived SCFAs play important roles in modulation of allergic reactions like asthma [28]. Zaiss et al. [28] also reported that transfer of '*H. polygyrus* bakeri modified microbiota' alone also could exhibit reductions in the severity of immune reactions and could prevent allergic outbursts.

The source of the SCFAs is the complex oligosaccharides present in host diet that are fermented by the bacteria to produce SCFAs. Interestingly, research shows that people from developing countries consume diets rich in fermentable fibers with higher concentrations of SCFAs being exhibited in their stools [12]. These people rarely suffer from allergies and show high incidences of helminth infections [29]. Based on these observations, Zaiss et al. [28] hypothesized that helminths-intestinal bacteria-diet may well interact in an evolutionarily conditioned concerted manner leading to immune-homeostasis both through direct (helminths-host-immune system) and/or indirect means (helminth-bacteria SCFAs host immune system). This area

needs further study because it provides important insights to the use of helminths or the helminths-induced microbiota alone or a mixture of both as therapeutic agents in prevention of allergic diseases.

Providing aid to life-cycle associated phenomena of the helminth parasite

Apart from immune control mechanisms, the gut bacteria have also been reportd to be closely associated with many important life-history stages of the helminth parasite. The classic experiments by Hayes et al. [30] revealed that the gut bacteria had important roles in the hatching of *Trichuris muris* eggs in the intestine of mice. They postulated that physical contact between the eggs and the commensal gut bacteria, *Escherichia coli* was essential for egg-hatching. Transwells of different pore sizes were used in their experiments and when the pores were small enough to prevent physical contact between *E. coli* and *T. muris* eggs, the eggs failed to hatch. This also demonstrated that bacteria-derived secretion probably played no role in the egg-hatching process because they could have easily diffused across the pores and bring about the hatching of eggs. According to Hayes et al. [30] the type 1 fimbriae of *E. coli* that binds the bacterial cells to the surfaces in a mannose-dependant manner may play an important role in egg hatching. They also observed that purified type 1 fimbriae could not stimulate the hatching process and addition of exogenous mannose molecules inhibited hatching. Other experiments using strains of *E. coli* that did not express the type 1 fimbriae also failed to induce hatching [30]. A series of experiments confirmed that the *E. coli* anchored (by type 1 fimbriae) around the opercula at the poles of the *T. muris* eggs and brought about the hatching [30]. However, this entire process requires an optimum temperature of 37°C [30]. Temperature is again an important factor because the egg hatching of *T. muris* becomes limited by two constraints: temperature and bacteria. These constraints prevent the hatching of the *T. muris* eggs outside the host body [30] and ensure the survival and transmission of the parasite from one host to the other. This is very important on the part of the helminth because it provides a kind of safeguard against its extinction (considering the worst possible outcome). *Salmonella typhimurium* could also bring about a similar mannose-dependant hatching [30]. However, neither *Pseudomonas aeruginosa* nor *Staphylococcus aureus* possess type 1 fimbriae yet could bring about the hatching of *T. muris* eggs [30]. This indicates that hatching mechanisms other than type1 fimbriae mediated process also exists [30].

Hayes et al. [30] however did not mention about the isolates of *T. muris* used in the experiments. Koyama [31] went a step further and performed the hatching experiments with all the three available isolates of *T. muris* eggs [E, E-J and S]. The bacteria used in the experiments were *E. coli*, *S. aureus* and *Enterococcus faecalis*. The experiments showed that *E. coli* and *S. aureus* could induce the hatching of E and E-J *T. muris* eggs under *in vitro* conditions while *E. faecalis* could not stimulate the same. Under the same conditions the S isolates were found to be unresponsive to bacterial stimulation [31]. It was also experimentally demonstrated that the egg hatching was suppressed (in E and E-J isolates) when the experiments were carried out in presence of an antibiotic (kanamycin) or when the bacteria used in the experiments were previously incubated at 4°C or fixed with formaldehyde [31]. However, differences in infectivity of E-J and S isolates were not observed [31]. From these experiments Koyama [31] concluded that: a) there must be some bacteria-independent in vivo hatching mechanism besides a bacteria-dependant one, b) the bacteria must be viable to bring about the hatching and c) the bacteria-mediated hatching of the helminths eggs was needed only for establishment of initial infection. The eggs after ingestion passes from the stomach to the upper part of the small intestine (by peristalsis) where the hatching takes place in about 30 minutes post infection [31]. However, this part of the small intestine harbours less of *E. coli* and *S. aureus* and more of yeasts, *Lactobacillus* and *Enterococcus* [32]. Thus the bacterial population alone may not be sufficient enough to bring about the hatching of *T. muris* eggs in 30 minutes [31]. This gives indication of some alternative bacteria-independent hatching mechanisms working under in vivo conditions thereby strengthening the hypothesis of Koyama [31]. Koyama [31] added that the eggs on reaching the upper parts of the small intestine become activated by some unidentified processes that facilitate their hatching. This activation though seems to be applicable to E and E-J isolates, the S isolates probably do not depend on bacterial stimulation [31].

Koyama [31] also stressed on the fact that the E and E-J isolates of *T. muris* have been maintained in the laboratories for a long period of time (E isolate since 1954 and E-J isolate since 1961) and repeated subcultures must have resulted in these isolates losing some of their original biological features. In contrast, the S isolate being comparatively recent (being maintained since 1989) must have retained some of its wild-type characteristic features [31]. These facts may also be a reason for the observed differences in their responsiveness to bacteria under experimental conditions [31].

In more recent works on egg-hatching, Vejzagić et al. [33] tried to study whether the bacteria-mediated hatching of *T. muris* and *T. suis* eggs followed the same or some different mechanisms. In their experiments *T. muris* and *T. suis* eggs were incubated under similar conditions with different strains of *E. coli* and gram positive bacteria (*Enterococcus caccae*, *Streptococcus hyointestinalis*, *Lactobacillus reuteri*, *L. amylovorus* and *L. murinus*). While *T. muris* eggs hatched with all the bacteria, *T. suis* demonstrated an overall vey low hatching [33]. Moreover, *T. muris* hatching was found to be inconsistent and highly variable with the gram positive bacteria [33]. Vejzagić et al. [33] explained the differences in these observations by shedding light on the fact that differences in the feeding behaviour of mouse and pig was the reason behind different microbial compositions of their guts and thereby the effect of the bacteria (taken in the experiments) varied on the hatching of *T. muris* and *T. suis* eggs since all the bacteria taken in the experiments were not representative of the common genera of the pig and mouse intestinal tracts [33]. These observations can further be interpreted by the fact that different helminths have evolved different mechanisms of egg-hatchings depending on the host-gut microbiota that is again a result of the long history of co-evolution. The advantages of using pig models in helminths-mediated therapy in human is based on the fact that pig and human gut microbiota is very similar [34] and *T. suis* eggs hatch in the human gut providing the basis for treatment of patients with immune-mediated diseases [33]. Experimental cross-infections of pigs with *T. trichura* gave positive results indicating a common bacterial stimulus for *T. suis* and *T. trichura* egg hatching [33]. Vejzagić et al. [33] have stressed on further study of roles of bacterial stimulus on cross-infections among different species of *Trichuris* so that they can be effectively in therapeutics for immune-related diseases.

Thus, from the foregoing discussion it is clear that there exists no universal mechanism of bacteria-mediated or bacteria-independent egg hatching mechanisms in helminths. Further, since the gut houses many bacteria together with yeasts, it is very difficult to elucidate the role of one particular bacteria species on the hatching of helminths eggs. Rather, a mixture of factors might be responsible with the hatching being facilitated by the presence of bacteria. This is also important from the viewpoint of alteration of the gut microbial population by the

intestinal helminths. Whatever may be, the helminths do exploit the gut microbial flora for their own benefit and the series of experiments in this field provide ample evidence in support of this. More such phenomena need to be discovered to understand the establishment of helminth infections in animal gut.

Augmenting the fecundity of helminths

The fecundity of the helminths has also been proposed to vary with the microbial diversity of the host gut. The worm burden was found to be higher (following low-dose infection by *Heligmosomoides polygyrus* bakeri) in conventional mice as compared to that of germ free mice indicating higher fecundity in presence of microbes in the gut [35]. Studies with *Nippostrongylus brasiliensis* [36], *Trichinella spiralis* [37] and *Ascaridia galli* [38] also gave similar results. Even more recently Hashimoto et al. [39] hypothesized that helminth mediated Th2 response (with respect to *H. polygyrus*) during the primary stages of infection leads to reduced number of eggs per gram of faeces, indicating a reduced fecundity. From the immunological standpoint, the dampening effect of the entire series of events between the bacteria and the helminth (and vice versa) may boost the fecundity of the helminth. This field needs more clarification.

Shift in the metabolome in the animal gut due to the presence of helminths and bacteria

The metabolic activities within the animal gut are to a large extent modified by the presence of helminths and microbes in the gut [7]. There are evidences that the metabolic activities are shifted in favour of both the helminth and the bacteria so that they may co-exist peacefully in the host gut. Li et al. [40] hypothesized that the mucosal injury caused by *Trichuris suis* in the gut of pigs led to an increase of microbial population like the Mucispirillum bacteria having strong predilections for the mucosal surfaces. This could cause increased mucous production which instead of eliminating the worms from the host gut could actually be protecting the helminths from the gastric juices and/or other strong proteolytic secretions in the host gut. This needs to be studied in detail.

Reports also show that carbohydrate metabolism of the animal gut is strongly impaired by reduction of cellulolytic and other carbohydrate utilizing bacteria due to the presence of helminths [41]. The dietary carbohydrates, their fermentability and physical properties, play important roles in the establishment of helminths infection, the distribution of the helminths in the intestine and the fecundity of the helminths within the host [42]. Reports show that carbohydrates that are readily fermentable result in a reduced fecundity and reduced growth of worms in the intestine but does not affect their establishment [43]. The morphology and mucin biosynthesis of the host large intestine is reportedly influenced by both helminths infections and dietary carbohydrates [41]. Metabolism of dietary carbohydrates is regulated by the intestinal microorganisms mainly in the hindguts of monogastric animals like pigs and humans [40]. The fermentation products like butyrate and acetates are absorbed by the intestinal epithelium acting as an energy-source and also serve to improve local epithelial cell function [40]. In pigs fed with fermentable carbohydrates, the SCFA concentration is greater in the large intestine with a reduction the luminal pH value which affects the intestinal microbial population [40]. Li et al. [40] in their experiments demonstrated that the number of open reading frames (ORFs) identified for carbohydrate metabolism processes were significantly reduced in *T. suis* infections [40]. 16S rRNA gene-based molecular identification techniques revealed that several bacterial genera with activities related to carbohydrate metabolism were drastically reduced because of infection [40]. These results indicated the impairment of the ability of the proximal colon microbiota for carbohydrate utilization [40]. Taking clues from these findings Li et al. [40] supported the used of diets rich in highly fermentable carbohydrates as a nematode control strategy in pigs earlier hypothesized by Petkevicius et al. [42]. The basic principle underlying this strategy is that the dietary carbohydrates would be fermented by the gut microbes providing energy supplements (in the carbohydrate metabolism impaired host gut) that may help in the repair of the epithelial damage of the colon and thereby enhance protective immunity, reduced inflammation, restore capacity for mucin biosynthesis and finally worm-expulsion through epithelial cell turnover [40]. It is a known fact that helminth infections are usually accompanied by malnutrition. Older works have focussed on direct roles of helminths to be a cause of malnutrition. These current studies develop the idea that the malnutrition can also be induced by helminths in an indirect bacteria-induced way (by suppression of carbohydrate utilizing bacteria). These results are important because in either case the establishment of a healthy gut epithelium is a pre-requisite for protection against helminth infections. Therefore Li et al. [40] has suggested the use of probiotics together with the helminths (when they are used as therapeutics) for maintenance of healthy gut epithelium.

There is also a dearth in lysine synthesis [44] and deficiency of sulphur containing amino acids like cysteine and methionine [45] in helminth affected individuals. Increase in oleic acid levels in the colon of pigs due to presence of helminths have been observed by Knoch et al. [46]. Oleic acid reportedly has antimicrobial properties [47].

These alterations of the metabolic activities in the host gut undoubtedly has a negative impact on host nutrition and their roles in altering the gut bacterial populations cannot be negated either, taking the antimicrobial action of oleic acid as an example. Though the impact of each and every metabolite on the gut helminth has not been deciphered yet, it may be assumed that in some way or the other the worm benefits from such a situation. This also needs to be studied. The argument cannot be restricted to the metabolic benefits of the worms alone; the immunological effects due to such metabolic shifts should also be taken into account. Wu et al. [10] reported an increased expression of the inflammatory genes, *c3ar1*, *cxcr2*, *ptgs2*, *ill3ra2* and *muc5ac*. The expression of *cxcr2* and *ill3ra2* has important roles in control of inflammation [48]. This well co-ordinated network regulating the gut homeostasis is a storehouse of secrets to be unravelled by further studies in this field.

Co-evolution of the microfauna and macrofauna in the animal gut

The animal gut has co-evolved with the microbes that are important determining factors of its proper functioning and elimination of the microbes may lead to dire physiological conditions together with a reduced protection against the entry of pathogenic bacteria [49]. Recent incorporation of the macrofauna as an extended component of animal guts has garnered interest in the co-evolution of the animal gut all the more. The gut has become tolerant to such non-resident outsiders by becoming more tolerant towards the molecules released by them. All these interactions reflect subtle forces of evolution working underneath for years. The metabolic shifts and immune suppression are not so simple and as one delves deeper, one finds that shifts in gene expression underlie such alterations. The concept of modulation of gene expression by the commensal gut bacteria has been proposed by Hooper et al. [50]. The evolutionary forces exerted by the gut microfauna and macrofauna over time in selection of certain immunoregulatory genes over the

other thus, cannot be neglected. Study of these co-evolutionary forces is important in understanding the growing potentiality of the parasites (their negative effects) and also their therapeutic uses (their positive effects).

Future Perspectives

The number of experiments performed in this field and the results obtained there from has given the researchers deeper insights into mainly the following thrust areas with futuristic implications

a. **Co-evolution and co-adaptation**

The gut has co-evolved with the micro and macrofauna with increased co-adaptation among the host, microbes and helminths (or other metazoan parasites). The concept receives strong support from the studies highlighting the increased incidences of inflammatory diseases in westernized countries due to eradication of gut helminths [51] leading to speculations that normal helminth infections are essential for a healthy gut. This has thus generated reduced pathogen virulence and reduced mortality rates of the hosts.

b. **New measures against helminth parasites**

The study of the three way interactions between the host, the gut microbe and the gut helminths has been found to give new ideas of helminth control. As suggested by Li et al. [40] if the hosts are given dietary supplements of carbohydrates or other metabolites (the normal functioning of which has been disrupted by the helminth), it helps in revitalising the gut mucosa and automatic elimination of the helminths. Though, this may have limited applications in case of the humans, it may have important application in the veterinary field especially in case of economically important animals.

c. **New biomarkers of helminth infection**

As already highlighted, the microbial composition of the gut gets altered due to the helminth. This alteration manifests itself by the presence of unsusual bacteria [40], greater proportion of common bacteria [40] or by presence of some metabolites in the faeces and/or urine [17]. These may be used as biomarkers for diagnosis of helminth infection that may be utilized for the humans and veterinary animals of economic importance.

d. **Use of helminths in therapeutics**

The 'hygiene hypothesis' emphasizes on the fact that reduced exposure to pathogens in the childhood due to improved sanitary habits have serious effects on the development of the immune system [52]. This results in the increased incidences of allergic reactions and immune related problems in the developed countries [52]. The use of helminths as therapeutics finds its base in the re-introduction of infectious agents like parasitic helminths with immunosuppressive properties as an agent of control for immune related diseases [52]. Use of two gastrointestinal nematodes, Trichuris sp. and *Necator americanus,* in this field have been studied extensively [52]. The embryonated eggs of *T. suis* (TSO) is an example of the raw material of the active pharmaceutical ingredient (API) in medicinal products are currently being explored for treatment of patients with immune-related disorders [33]. Trichuris infections are known to elicit type 2 immune responses in the host that downregulates type 1 and type 17 immune responses generally associated with many autoimmune dieases [52]. This immune downregulation forms the basis of treatment using helminths. Giancomin et al. [52] opined that since the intestinal microbiota is reportedly disturbed in many immune disorders and since the gastrointestinal helminths and the microbes occupy the same environmental niche, the proper understanding of the interactions between the helminths and the microbe is very important for the proper use of helminths as therapeutics. The hookworm, *Necator americanus* has been suggested to be advantageous over *T. suis* as therapeutic agents since *N. americanus* can survive for longer periods in the human host and need not be repeatedly administered [52]. Moreover, the risks of transmission of *N. americanus* infections is also reduced under current highly sanitary-conscious environment [53].

Use of helminths in allergic diseases because of their immune-suppressive effects (at least in some species) has been well documented by Maizels et al. [54]. Treatment of IBD in murine models using *H. polygyrus* [7] or *T. suis* in pig models [55] has important medical applications. Treatment of autoimmune diseases by helminth infections has also been proposed by Leung and Loke [5]. Treatment of colitis with *Trichuris trichiura* infections in macaques [56] also gives valuable application of such studies.

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