



# Human ascariasis, an evolutionary complex relationship between host and parasite

**Ascariasis humana, una relación evolutivamente compleja entre el huésped y el parásito**

**Ascaridíase humana, uma relação evolutivamente complexa entre hospedeiro e parasito**

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## Abstract

Ascariasis is a debilitating parasitic disease that has decimated the health of hundreds of thousands of human beings, especially in developing countries. The lack of adequate prophylaxis of the parasite associated with an increased risk of immunological disease is a challenge mainly in developed countries where the prevalence of this parasite is very low. The evolutionary relationship and mechanisms by which the parasite was able to colonize and establish itself in human hosts have not yet been unraveled. In addition, the host immune response mechanisms to eliminate or control the parasite are not fully understood. Understanding these immunological mechanisms (proximates) will allow establishing better medical treatments for diseases related to the positive effects of parasitosis, thus also avoiding the undesirable effects of the parasitosis itself.

**Keywords:** Evolution, Ascariasis, Zoonosis, Ascarid therapy, *Ascaris suum*, *Ascaris lumbricoides*.

## Resumen

La ascariasis es una enfermedad parasitaria que diezma la salud de cientos de miles de seres humanos, especialmente en los países en desarrollo. Sin embargo, la falta de profilaxis adecuada del parásito, asociado con un mayor riesgo de sufrir enfermedades inmunes, es un problema principalmente en los países desarrollados donde la prevalencia del parásito es muy baja. La relación evolutiva y los mecanismos por los cuales el parásito fue capaz de colonizar y establecerse en el huésped humano aún no se han descifrado. Del mismo modo, los mecanismos inmunes (proximales) de respuesta del huésped para eliminar o controlar el parásito no se entienden completamente. La comprensión de estos mecanismos nos permitirá establecer mejores tratamientos médicos, evitando efectos indeseables y más enfocados en el control de la parasitosis, así como para la terapia de estas enfermedades inmunológicas asociadas con la relación huésped-ascárido.

**Palabras clave:** Evolución, Ascariasis, Zoonosis, Terapia de Ascaridios, *Ascaris suum*, *Ascaris lumbricoides*

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### Resumo

A ascariíase é uma doença parasitária que dizima a saúde de milhares de seres humanos, principalmente nos países em desenvolvimento. No entanto, a falta de profilaxia adequada do parasito, associada ao aumento do risco de doenças imunológicas, é um problema principalmente em países desenvolvidos onde a prevalência do parasito é muito baixa. A relação evolutiva e os mecanismos pelos quais o parasito foi capaz de colonizar e se estabelecer no hospedeiro humano ainda não tem sido decifrados. Da mesma forma, os mecanismos de resposta imune do hospedeiro para eliminar ou controlar o parasito não são totalmente compreendidos. A compreensão desses mecanismos permitirá estabelecer melhores tratamentos *médicos*, evitando efeitos indesejáveis e mais voltados para o controle das parasitoses, bem como para a terapia dessas doenças imunológicas associadas à relação hospedeiro-ascarídeo.

**Palavras-chave:** Evolução, Ascariíase, Zoonoses, Terapia Ascarídea, *Ascaris suum*, *Ascaris lumbricoides*

### Introduction

Ascariasis is a disease in humans caused by ubiquitous gastrointestinal parasites known as *Ascaris lumbricoides* and occasionally also by *Ascaris suum* (Leles et al., 2012; Loreille and Bouchet, 2003). Ascariasis is one of the most widespread diseases worldwide, especially in tropical and subtropical geographical areas. It is estimated that between 1.2 and 1.5 billion people are affected, with 100-200 million individuals (mostly children) showing clinical symptoms. Poverty, lack of drinking water, poor sanitary and hygienic conditions are among the factors associated with its high transmission in regions with high prevalence (Betson et al., 2014; Dold and Holland, 2011; Loreille and Bouchet, 2003; Peng y Criscione, 2012). Ascariasis is considered a neglected tropical disease, among other parasitic diseases, prevalent mostly in developing countries (Dold and Holland, 2011).

*Ascaris lumbricoides* Linnaeus, 1758 and *Ascaris suum* Goeze, 1782 are morphologically indistinguishable (Betson et al., 2014) and have a genetic difference of only six nucleotides in their ITS-1 segment. Although classified by some authors as two distinct species, they are taxonomically very close to each other (Leles et al., 2012; Peng and Criscione, 2012). For example, both species have a monoxen-direct transmission life cycle (Criscione et al., 2007; Dold and Holland, 2011; Roberts et al., op. 2009) and identical disease mechanisms.

Economic losses associated with ascariasis can be quantified by counting disability-adjusted life years (DALYs = 10.5 million) (Barendregt, 2002; Dall' Orso et al., 2014). If we add the high morbidity rate associated with clinical diseases (~122 million cases/year), this disease has profound effects on both the public health system and the local economy (O'Lorcain and Holland, 2000) given treatment costs and the loss of working hours. On the other hand, some evidence suggests that this parasitosis may have beneficial effects, especially in reducing the incidence or development of immune-mediated diseases (Briggs et al., 2016; Correale, 2014). It has been hypothesized that this apparent positive effect in infections is related to a host-parasite evolution (Parker and Ollerton, 2013). This situation leaves a gap regarding the most appropriate treatment, the development of new therapies, and the control over this parasitosis in humans and animals based on evolutionary medicine.

### When *Homo sapiens* met *Ascaris lumbricoides*:

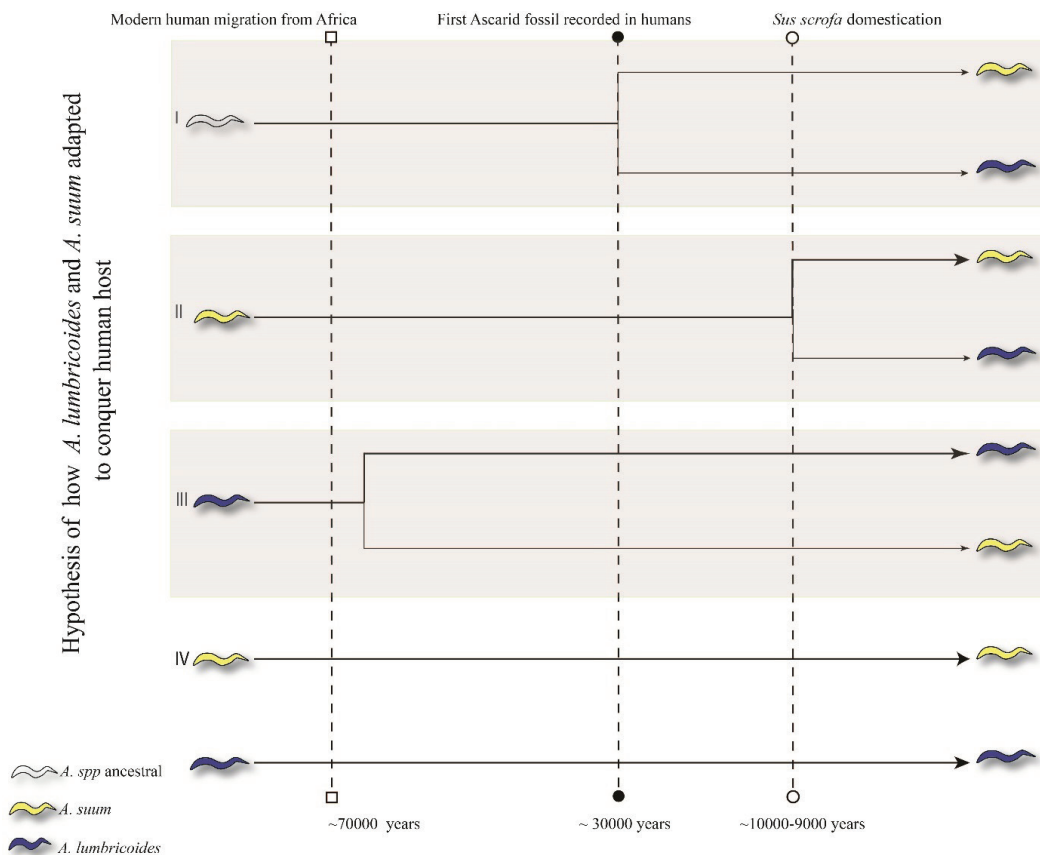
Several hypotheses attempt to explain the evolutionary relationship between *A. lumbricoides* and *Homo sapiens* (Criscione et al., 2007; Leles et al., 2012; Loreille and Bouchet, 2003). A very close evolutionary link has been suggested between *A. lumbricoides* and *A. suum* given their genetic similarity, life cycle resemblance, and cross-infections to humans and pigs (Loreille and Bouchet, 2003; Nejsun et al., 2012). Fossil records of ascarids



in humans date back to around 30,000 years ago. However, since these records are inconsistent, it cannot be ensured that they are unique to *H. sapiens* given the possibility of contamination with eggs of ascarids of other mammals (e.g. *Ursus speleacus*) (Leles et al., 2012). Certainly, pig domestication, linked to the emergence of ascariasis in humans, has not yet been fully elucidated. Palaeontological evidence suggests that pigs diversified into different groups: in the Americas in the late Eocene (34 million years ago) and in Europe, Asia, and Africa in the Oligocene (23 million years ago). On the other hand, large hominids emerged between 24 and 16 million years ago, followed by hominins who diverged from a common ancestor between 9 and 7 million years ago (Dunsworth, 2010). This suggests that humans and wild pigs coexisted in the same environment for a long time, being an important food source for early hunter-gatherers in Eurasia (Leles et al., 2012), thus opening up the possibility of ascariasis spillover through close contact between these two species.

Four hypotheses have been suggested for the origin of *A. lumbricoides* (Figure 1). The first hypothesis proposes that both *Ascaris* spp shared a common ancestor, possibly before the domestication of pigs (*S. scrofa*) around 10,000-9,000 years ago in the Near East (Larson et al., 2005; Rito et al., 2019). However, the lack of evidence of ascariasis in great apes and the low diversity of *Ascaris* in Old World primates place this hypothesis in a weak position (Leles et al., 2012).

**Figure 1:** Suggested *Ascaris lumbricoides* lineage evolution hypothesis towards new human host.





The second hypothesis states that *A. lumbricoides* derives directly from *A. suum* through an allopatric speciation event favoring host shift (pig to human) and that the current pig *Ascaris* is its persistent ancestor from the former (Liu et al., 2012). This origin has been suggested in the Neolithic, when pig domestication took place and, consequently, human populations increased, allowing the maintenance of the endemic cycle through epidemics (threshold host density) (Holt et al., 2003). Furthermore, at least two divergence events have been proposed for such parasite colonization, the first occurring ~15,000-2,500 years ago based on mitochondrial phylogeny (origin of haplotype cluster C, exclusive in European pigs) and a second more recent event, ~1,800-300 years ago (origin of haplotype clusters B and A, predominant in pigs in China and Africa) (Betson et al., 2014; Nejsum et al., 2017). Multiple successful host colonizations in different areas associated with human migrations suggested that such human populations might have been the starting point for the establishment of the definitive host shift (Zhou et al., 2011). This idea is based on the comparison of the evolutionary process that *Trichocephalus trichiurus* in humans originated from *Trichuris suis* (pig definitive host). However, this parallelism loses strength when comparing the criteria used for *Trichocephalus trichiurus*, as these hardly fit the criteria for the origin of *A. lumbricoides*. For example, *Ascaris* spp. are not very diverse in Old World primates, contrary to *Trichuris* spp. The higher rate survival of *A. lumbricoides* in humans (long-lived) suggests a better ecological adaptation to the human host compared to *A. suum*, which contrasts with reports on *T. suis* and *T. trichiurus*. Finally, the absence of robust paleontological material confirming the existence of *A. lumbricoides* in human settlements prior to pig domestication differs from the evidence for *T. trichiura* (Leles et al., 2012; Loreille and Bouchet, 2003). The lack of consensus on the natural ability of *A. lumbricoides* to effectively infect pigs (only with high parasite loads of *A. lumbricoides*) and the inability of *A. suum* to reach a mature stage in the human host are the most relevant arguments weakening this hypothesis (Leles et al., 2012).

Another possibility is that *A. suum* derived directly from *A. lumbricoides*, the former being the most recent species. Under this hypothesis, an allopatric event occurred, but from humans to pigs, which is debated based on the long association between early hominins in Africa and subsequent early human migrations. The spread of these ascarids to new geographical areas simultaneously occurring with pig domestication would provide the opportunity to jump to the new host species, but the absence of fossil records to support this idea weakens this third hypothesis (Betson et al., 2014; Leles et al., 2012; Loreille and Bouchet, 2003).

The hypothesis that both species (*L. lumbricoides* and *summs*) are conspecific is based on the low genetic divergence in several genetic markers (ITS1 of the rDNA) between the two species (Cavallero et al., 2013; Criscione et al., 2007; Leles et al., 2012). Experimentally and naturally, cross-infection between *A. suum* and *A. lumbricoides* has been achieved in the opposite hosts, thus completing the cycle in both cases. However, some authors have not been able to complete the *A. lumbricoides* cycle in pigs, arguing that very high parasite loads are required to achieve this infection in this species (Easton et al., 2020). This contradiction in this type of experimental infection has not allowed for a consensus on the true transmission capacity between these species. In addition, this hypothesis contradicts those who defend that both species are really one.

The argument to classify them as a single species is based on the low genetic composition plus their little morphological differentiation between these two nematodes, especially when they share the same geographical location. But others argued that this short difference in genetic makeup is enough to classify these species as divergent species (Leles et al. 2012). Betson et al. molecularly density analyzed ascarids samples from humans and pigs from around the world (mDNA, microsatellite markers), concluding that there are less than 10%



marked differences between these two species worldwide, which was not sufficient for other authors (Betson et al., 2014; Dold and Holland, 2011; Easton et al., 2020; Leles et al., 2012) to classify each of them into two distinct species. More recently DNA sequencing of the complete mitochondrial genome confirmed the existence of the previously described haplotype clusters (A, B, and C) using the mitochondrial marker *cox-1* (Betson et al., 2014; Cavallero et al., 2013; Nejsun et al., 2012; Nejsun et al., 2017).

### Genetic evidence of speciation:

It is more complicated to clarify whether these two species are assigned the status of a single species or conversely two different species using molecular markers to achieve the identification of the genotype. There are two haplotypes, one related to humans and the other to *Ascaris* in pigs. Anderson et al. showed that the mtDNA allozyme allele divergence in both clades is around 3-4%, showing that each of them has independent vital cycles, and, as a result, they are two different species (Anderson et al., 1993) a difference that was eventually corroborated by other authors using more accurate molecular markers (Betson et al., 2014; Peng and Criscione, 2012; Zhou et al., 2011).

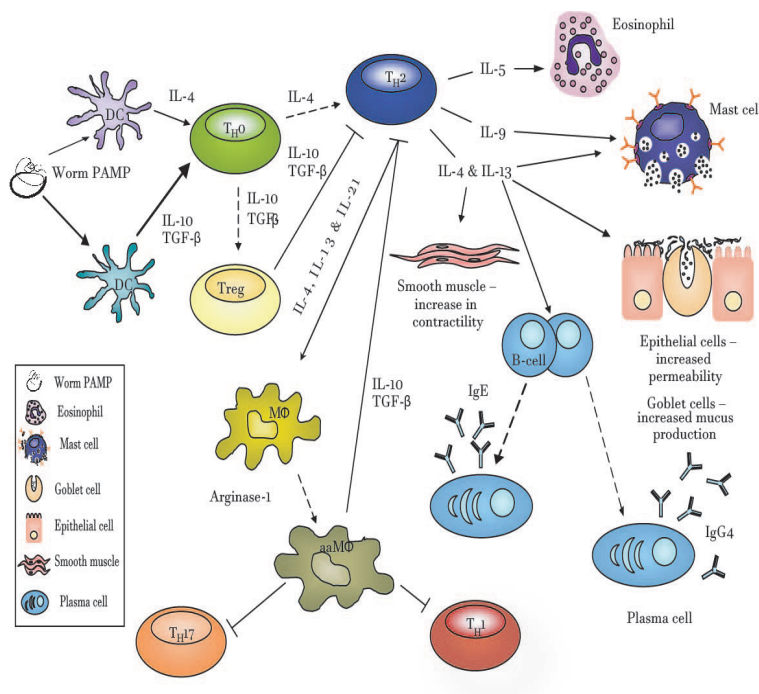
Cavallero et al. used restriction fragment length polymorphism to identify the nuclear ITS (ITS1, 5.8S, ITS2) region as a target for the pig and human *Ascaris*-specific marker. These analyses identified five parasitic genotypes. Genotype 1 was associated with humans, genotype 2 with pigs, while the other genotypes were found in both species. The sharing of genotypes between these two species would be explained by an evolutionary process such as introgression or retention of ancestral polymorphisms (Cavallero et al., 2013; Liu et al., 2012; Nadler and Hudspeth, 2000; Peng et al., 2003; Zhou et al., 2011). This means that, when hybridization between close relatives or when sister taxa diverge, they need an extended period of time to be truly genetically distinguished from each other (Anderson, 2001).

However, this marker (ITS-1) has a high intra-individual variability, which hinders its use as a reliable diagnostic marker when applied to close relatives species (Anderson, 2001; Leles et al., 2012). Given that the complete sequencing of the mitochondrial genome (mtDNA) of *A. lumbricoides* shows a 1.9% difference with the *A. suum* genome, it is considered a single species (Leles et al., 2012; Liu et al., 2012). Finally, the phylogenetic relationship of the ascarids established by Nadler and Hudspeth (Nadler y Hudspeth, 2000) using a parsimony analysis between morphology and genetic markers concludes that both species are sister taxa given the short pairwise distance, and that the most likely recent common ancestor is *Parascaris equorum* (equine ascarid) (Leles et al., 2012; Nadler and Hudspeth, 2000). Interbreeding between *A. suum* and *A. lumbricoides* has been observed in both China (7.8%) and Guatemala (4%) from the 23 microsatellite markers, but not in other sympatric geographic areas for these two sympatric species. Although the existence of inbreeding is confirmed, the fitness of these hybrids appears to be very low; otherwise, the population structure in the hybrid would have been established in these populations (Betson et al., 2014; Holland and Smith, 2005; Zhou et al., 2011). However, the inconsistency to establish a clear separation or non-separation between these two species is related to the selection of molecular diagnostic markers, by not including several sequences of loci, which can be achieved by means of next generation sequence methods and increasing the number of samples from different geographic areas in the world (Anderson, 2001; Easton et al., 2020). In this way, the difference between these species could be established in an adequate and definitive manner (Holland and Smith, 2005; Leles et al., 2012; Peng and Criscione, 2012).



### Disease or proximate mechanisms:

Regardless of the mechanism used to establish this parasitic relationship between *H. sapiens* and *A. lumbricoides*, the question to be elucidated here is whether *A. suum* and *A. lumbricoides* are distinct species. What is clear is that the association over time has generated evolutionary drivers of parasite colonization and the host's antagonistic response against this colonization. In *Ascaris* spp. infection (Girgis et al., 2013), the host uses both innate and adaptive responses as a defense against this invasion. The former response is expressed by granulocyte cells, macrophages and active metabolites (innate), while the latter uses an adaptive response with a set of regulatory cells, expressed by Th<sub>2</sub>-T<sub>reg</sub> cells, Regulatory B (B<sub>reg</sub>) (McSorley and Maizels, 2012; Turner et al., 2005). The establishment of intestinal resistance in the host is directly associated with this Th<sub>2</sub> response, which has been demonstrated in murine models (Turner et al., 2005). Once larvae enter the bloodstream, an immune response is initiated by dendritic cells (DC) through Toll-like receptor (TLR) recognition of parasite antigens (cuticulin proteins, ascarosides and a plethora of protein structures, such as ABA-1-NPAs) and by presentation of MCH II antigens, which induce differentiation of CD<sub>4</sub> Th<sub>2</sub> cells. These promote the humoral secretion of several cytokine-interleukins (IL-10, IL-4,5,9 and IL-13) (Figure 2). This complex mechanism causes Th<sub>2</sub> to activate, amplify, and maintain key innate effectors such as mast cells, basophils, eosinophils, and macrophages alternatively activated to eliminate the parasite. The expression of phenotypes through polymorphic genes of the effector subset of Th<sub>1</sub> (IFN-γ) and Th<sub>17</sub> cells via activation of DC presentation is responsible for the most relevant mechanisms associated with inflammation, pathology and parasite expulsion, the latter especially at the level of the intestinal lumen (Turner et al., 2005).



**Figure 2:** Innate and acquired pathways (cells and proteins involved) in establishing an anti-helminth immune response and the proximate mechanisms involved in Th<sub>2</sub> response against helminths. Modified from Jackson et al. 2009 (permission granted by the authors). Pathogen-associated molecular patterns (PAMPs), dendritic cells (DCs), regulatory T cells (Treg), T-helper cells (Th), activated macrophages (aaMφ), naive Th cells (Th<sub>0</sub>), transforming growth factor (TGF)-β, Damage-associated molecular patterns (DAMPs), eosinophil-derived neurotoxin (EDN), Immunoglobulin type (IgX), Interleukin type (ILX) modified with permission of the authors (McSorley and Maizels, 2012).



These responses are highly antagonized by Th<sub>2</sub> cells (a source of IL-4) and T<sub>reg</sub> cells (a source of IL-10 and transforming growth factor beta (TGF-β) in the case of helminthiasis) (Holland and Smith, 2005). The parasite load in the host is not necessarily related to a higher pathology. Owing to the response of the immune system is paradoxical, as there are asymptomatic individuals with a strong regulatory response while chronic disease is observed in affected animals with low levels of infection. The asymptomatic type response is mainly regulated by IL-10 and TGF-β (CD4+T<sub>reg</sub> 1), down-regulating Th<sub>1</sub> and Th<sub>2</sub> responses, whereas the acute type response is largely regulated by Th<sub>2</sub> suite cells and granulocytes (Kayes, 2005; Maizels and McSorley, 2016).

On the other hand, the humoral response is associated with activated B cells (B<sub>reg</sub>) in the presence of *A. lumbricoides* antigens or alternatively by Th<sub>2</sub> activation. This B effector expresses immunoglobulins of various types, notably IgE and IgG (Holland and Smith, 2005). These appear to offer some degree of protection by initiating an allergic-type reaction against the *Ascaris*, as well as Th<sub>2</sub> amplifiers with effector mechanisms to clear the infection. In mouse models infected with *Heligmosomoides polyurus*, there is a suppression of the immune system that prevents encephalomyelitis and allergies in mice transplanted with B<sub>reg</sub> cells from parasitized mice (Jackson et al., 2009). This effect is attributed to IL-10 production and indirectly to T<sub>reg</sub> cell activation. These cell types are the main down-regulators of the immune system in the presence of helminths (Dold and Holland, 2011; Jackson et al., 2009; Maizels and McSorley, 2016). T<sub>reg</sub> cells are associated with a tolerance effect, triggering a series of linked events that promote, reinforce, and maintain Th<sub>2</sub> pathways during infection (Allen and Maizels, 2011). The main cell phenotypes responsible for that tolerance effect are “natural” T<sub>reg</sub>, which express the cytokine FoxP3 after thymus selection, “induced” T<sub>reg</sub>, which switch to Foxp3 at the peripheral level, and Foxp3-type 1 regulatory cells. All these cells are the major expression of IL-10 and TGF-β at different levels (Girgis et al., 2013; Holland and Smith, 2005; McSorley and Maizels, 2012). These two cytokines are the principal regulators of the host response against helminths, as they enable the switch from IgE (pro-allergic/inflammatory) to IgG (non-inflammatory) isotype in chronic infections. Evidence suggests that helminths are partially responsible for this mechanism, as the administration of an anthelmintic treatment to affected patients causes a dramatic drop in circulating IgG level (Jackson et al., 2009). This would explain, to some extent, this apparent contradictory response of the host immune system to the parasite (Maizels and McSorley, 2016).

Finally, susceptibility to *A. lumbricoides* has been described in human family groups and may be associated with genetic factors. Studies in Nigerian children’s populations, for example, suggest an association between susceptibility to the parasite and the presence of major histocompatibility complex (MHC) resistance alleles. However, it is difficult to select which genes to study since there is not a clear identification of which are the ideal ones, leading to contradictory results such as variation on genetics and environmental factors associated with the worm burden (Williams-Blangero et al., 1999). A longitudinal study that followed subjects over a two-year period showed that in a population of 1261 people from Nepal with a known pedigree, a strong relationship between genetic factors (genes: IL7 and activated B-cell factor 1), as well as susceptibility to *A. lumbricoides*, was established (see details in Williams-Blangero *et al* 1999). This robust study demonstrated a correlation between the phenotype of human groups and helminth egg load and concluded that between 30% (p=0.0016) and 50% (p<0.0001) of the variation in worm burden was due to genetic factors. This was the first study to establish the importance of host genetic factors in determining parasite load, demonstrating the urgency of determining which genes are responsible for causing this susceptibility (Williams-Blangero et al., 2013).

### **Parasite evasion mechanisms:**

Vertebrates have evolved a complex immune response to defend themselves against various micro and macroparasites, which focuses on keeping the parasite under control. This demonstrates the complex process of co-evolution between hosts and parasites (Schmid-Hempel, 2009). Throughout the evolutionary history between host and parasite, the immune system has carried out an innate immune response according to the type of threat (pathogens). However, roundworms have evolved several mechanisms to evade or regulate the host immune system to ensure their coexistence. *Ascaris* infections are usually chronic, with a low pathogenic effect on the host (lower growth rate and lower capacity for damage in target organs), which implies a level of adaptation in the process of co-evolution with their host (Jackson et al. 2009). A high prevalence of these parasites can be seen in humans and pigs in endemic zones (Dold and Holland, 2011). Some of the strategies of ascarids to remain in their host are through the expression of genes encoding molecules such as TGF- $\beta$ -like ligand, glycoproteins, phosphorylcholines together with ES-62 N-type glycans by down-modulation, subversion or evasion of the host immune system (Girgis et al., 2013). These mechanisms, although not yet well understood, allow the parasite to complete its life cycle by reaching sexual maturity, reproducing in its host and leaving offspring for the species to survive.

### **Immune tolerance trade-off:**

The concept of co-evolution is reflected in the host-parasite's own mechanisms and their constant adjustment and imbalance as an evolutionary response to a strategy aimed at reinforcing the immune defense system (receptors, molecular mediators, signaling pathways) over time (Dold and Holland, 2011; Jackson et al., 2009; Maizels and McSorley, 2016). Thus, vertebrates developed an immune system that anticipated encounters (due to the ubiquitous nature of helminths) with parasites that stimulated the Th<sub>2</sub>/T<sub>reg</sub> response (Jackson et al., 2009; Maizels, 2009).

The complex response of the immune system to helminths echoes the diversification of methods and redundancy in some of them to develop and strengthen mechanisms of resistance to the presence of ascarids. For example, the Th<sub>2</sub>-mediated response is mimicked or reinforced by a wide array of helper cells in the innate response by secreting chemical mediators that further stimulate the Th<sub>2</sub> response in helminth infections, or by overlapping the cytokine-producing function of other cells (especially non-B or T cells to perform CD4<sup>+</sup>Th<sub>2</sub>-like functions) (Allen and Maizels, 2011; Maizels and McSorley, 2016). There is no evidence that nematode expulsion or death occurs exclusively by granulocyte action; rather it is possibly associated with the energetic cost, as the activation of this innate response and the associated high cost of controlling this tissue pathology overcomes the benefit (Maizels, 2009; Turner et al., 2008). It seems that in this co-evolutionary process between host and parasite, the host established a mechanism to apply a more reasonable strategy. This is based on resistance-tolerance to the parasite, ensuring the maintenance of immune homeostasis at an acceptable cost-benefit to achieve optimal fitness. These two antagonistic processes (resistance-tolerance) occur simultaneously in the host due to this homeostatic balance. Resistance is expressed in the mechanisms through Th<sub>2</sub> cells (immune regulation, repair, and anti-parasitic effect), while tolerance is expressed in complex mechanisms targeting the active suppression of autoreactive T<sub>reg</sub> cells mediated by T<sub>reg</sub> cells as an essential mechanism of self-tolerance and B-cell action (Allen and Maizels, 2011; Johnston et al., 2014). *H. sapiens* populations possess extensive polymorphism in immune-associated genes



that have evolved to positively select those alleles that express an “optimal” response to the parasite (tolerance-resistance). Interestingly, parasite tolerance may be favored in children born to parasite-infected mothers (uterine tolerance), being more susceptible to infections at a younger age by showing a lower T-cell response to parasite antigen than those born to parasite-free mothers (Maizels and McSorley, 2016). Unfortunately, the ineffective memory response of the host immune system to the parasite is a product of this damping effect. This has been demonstrated in mouse models of helminth infection and in human ascariasis, where after total parasite elimination by anthelmintic therapy, subjects are susceptible to parasites in the absence of a memory immune response (Maizels, 2009). In addition, the immune system is unable to maintain a  $Th_2/T_{reg}$  response and a downmodulated cytokine production (IL-10) in the absence of the parasite stimulus, which may explain some of the increased incidence of allergies and autoimmune diseases in developed countries (Jackson et al., 2009; McSorley and Maizels, 2012). High single nucleotide polymorphism (SNP) frequencies were determined in an analysis of ~100 human interleukin genes. In geographic areas with a high helminth presence, these loci show high variability in encoding cytokines for both innate and adaptive responses (Maizels, 2009). The low incidence of immunopathology in endemic countries with high prevalence of helminths (including ascarids) is associated with a genetic locus that increases the probability of developing tolerance against the parasite in developing countries. This selection is evident, for example, in a non-coding variant allele of the STAT-6 regulatory transcript that is part of the IL-4 pathway, which is associated with increased incidence of asthma due to the absence of infection by ascariasis and reduced susceptibility against *A. lumbricoides* in humans (Maizels, 2009). This evidence suggests that there was an evolutionary mismatch in the compensation trade-off.

### **Ascarid strategy:**

The immune system has evolved by constant helminth infestation; as a result, helminths (including ascarids) have counteracted the host, evolving damping processes rather than evading or deactivating the host immune system response (McSorley and Maizels, 2012). In this trade-off, the parasite reinforces the host’s strategy to tolerate ascarids by producing molecules that mimic endogenous host mechanisms, which, in turn, control immunosuppression. For example, blockade of TLR receptors, which are used by other molecules such as lipopolysaccharides as an inflammatory response, is a phenomenon observed in mice (*Apodemus sylvaticus*), which are infected by *H. polygyrus* under natural conditions (Maizels, 2009). This may partly explain the parasite’s strategy to stimulate host tolerance. Although nematode defense mechanisms are still poorly understood, it is known that the helminth cuticle can stimulate a complement-mediated inflammatory response via the alternative pathway. The complement antagonistic response of the parasite occurs through the secretion of molecules on the surface cuticle ES-62 (Coakley et al., 2016). In turn,  $B_{reg}$  cells are part of this down-regulation, by being directly stimulated by helminths and expressing IL-10, a phenomenon observed in rodent models (McSorley and Maizels, 2012).

### **Anthelmintic resistance:**

In human ascariasis, strategies to control endemic populations are to reduce parasite intensity through improved health conditions, environmental sanitation, health information, and anthelmintic chemotherapy (Dold and Holland, 2011; Holland and Smith, 2005). Benzimidazole derivatives (albendazole) bind to



$\beta$ -tubulin and block tubulin polymerization, thereby destabilizing helminth microtubules. These drugs have been used in mass drug campaigns in endemic areas such Nigeria or Nepals (Leung et al., 2020); however, this could lead to their resistance (Diawara et al., 2009; Holland and Smith, 2005; Krücken et al., 2017).

Evidence of rodent models with human parasites have identified the emergence of helminths with resistance to these drugs, which is associated with resistance alleles (F167Y, E198A, F200Y) (Churcher and Basanez, 2008; Dilks et al., 2020). In *Trichocephalus trichiurus*, resistance is associated with a mutation in the SNP responsible for a substitution of the amino acid Phenylalanine for Tyrosine at codon parasite 200 TAC mutant type SNP. It is unclear whether this resistance is recessive, semi-dominant, or dominant (Diawara et al., 2009). The question, therefore, arises as to whether there will be a positive selection force of these genes in populations under constant pressure from this drug as a parasite evolutionary mechanism (Churcher and Basanez, 2008). Mathematical modeling showing the global decrease in parasite load on the host evidences the negative impact of density-dependent processes on natural selection on resistance genes. Findings suggest that the use of anthelmintic therapies leads to parasite depletion in the host, releasing the pressure of negative density-dependent mechanisms on the surviving parasites (Churcher and Basanez, 2008). This increases the likelihood of transmission of resistance alleles in the progeny. In addition, the possibility of host-to-host transmission and the generation of hybrids of *A. suum* and *A. lumbricoides* may lead to a process of introgression with the expression of a deworming resistant phenotype. This may favor an increased virulence of the parasite or gene flow and thus the transfer of resistance alleles (Betson et al., 2014; Criscione et al., 2007).

### Horizon:

Multiple rodent models of helminth parasitosis have proven the association between the parasite and its down-regulatory mechanism of the immune system, leading to an antigen-specific anergic stage, which results in a suppression of symptoms in pathologically induced mice (Zhou et al., 2011). Protective effects of *A. lumbricoides* and other gastrointestinal helminths against multiple sclerosis have been described in accidentally infected sclerotic patients (Correale, 2014). This damping effect does not always have positive consequences for the host. Host compensation can generate negative effects (Figure 2) over the interaction of the nematode (for example *Schistosoma haematobium*) with the host immune response. That effect is a bioproduct that contributes to a positive carcinogenic outcome favoring the growth of cancer cells through the production of cancer promoters by the parasite as a result of this damping or by reducing the efficacy of vaccines or increasing susceptibility to other infections or other microparasites such as the influenza virus (Casado-Maestre et al., 2011; Johnston et al., 2014; McSorley and Maizels, 2012; Schneider-Crease et al., 2021). In this context, the proximity mechanism, as understood from the evolutionary point of view, can give a new horizon for a better mechanism in disease therapies.

### Therapy drawback:

Much of the controversy surrounding live parasite therapies as a treatment for autoimmune conditions and associated diseases in humans is based on findings associated with the paradoxical effects of this relationship (old friend and hygiene hypothesis) (Frew, 2019). On one hand, their true efficacy is questioned, as they are based on animal-parasite models that do not reflect the genuine response expected in humans. On the



other hand, studies show little or no efficacy of parasites in reducing these type of diseases (Briggs et al., 2016; Wammes et al., 2014). Multiple studies emphasizes, rather, the high risk that exists with the parasitic loads used and the pernicious effects of this iatrogenic parasitosis (Briggs et al., 2016). Even more critical is the fact that several studies suggest a rather opposite effect to the proposed protective result, lighting the increased allergic susceptibility of the host due to helminthic infections (Wammes et al., 2014). However, this conflicting evidence is based on the results of studies with a certain degree of bias due to the animals or mathematical models used. This stresses the need for a more accurate model. There are multiple factors such as burden, host genetic, parasitosis chronicity, or mix factors that may mask the true beneficial effect of helminths and their evolving host-parasite relationship. Future research should include aspects such as the role of parasite ecology and the difference in cultural and epigenetic patterns to try to give a more accurate explanation of the benefit or this type of future therapy (Kondrashova et al., 2013; Wammes et al., 2014; Yang y Schwartz, 2012). Prioritizing studies that focus on parasite bioproducts (proteins, lipids, etc.) that could modulate the immune-metabolic pathway for future and safer therapies should be the aim of new experiments and treatments (van Kruiningen and West, 2005).

## Final remarks

Parasitic diseases have been associated with human beginnings, and this relationship between both host and parasite has developed over time. Evolutionary medicine believes in a strong link between evolutive and proximate mechanisms, giving an accurate explanation for the tradeoff associated with diseases and the relationship between pathogens (macro and micro parasite) and their hosts. Ascariasis in humans caused by *Ascaris lumbricoides* or *A. suum* is a good example in which both mechanisms can be explored and illustrates the effect of how such mechanism should be better understood under the evolution perspective.

Using an evolutionary approach, immunological mechanisms responsible for the damping process and host-parasite interactions could be understood in detail and give guidance for better therapies to avoid negative evolutionary mismatch. The identification of precise genetic variants could develop future therapies, including the manipulation of disease mechanisms to control autoimmune diseases and allergies, the prevention of allographic rejection, and a better targeting of control methods of this parasitosis in the near future (Johnston et al., 2014).

A better understanding of how evolution is part of modern medicine is paramount to having a more advanced human and animal medicine, without losing sight of the powerful influence that evolution has on health.

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