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Parasites: the future of biotherapy

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Abstract

Biotherapy targets molecules that alter the immune response. It involves a plethora of organisms known to alter the course of myriad diseases and ailments. Many of these diseases can be life-threatening to the humans and animals, and exhibit resistance to available antimicrobial medications. To address such ailments, traditional and modernized therapies that target specific molecules responsible for altering the immune response are currently being explored. Such therapies utilize various organisms that are known to impact the progression of numerous diseases and disorders. Diseases caused by certain organisms can also alter the courses or outcomes of other diseases. Biotherapies such as helminth therapy, maggot debridement therapy, and hirudotherapy use parasites (roundworms and flatworms), arthropods (maggots), and leeches (annelids), respectively, as potential biological therapeutic sources to treat autoimmune and other chronic diseases. Where conventional medicine fails, these traditional-turned-modern alternative therapies can serve to boost the health prospects of patients who are vulnerable to the misery and pain inflicted by their ailments. Patients dealing with these circumstances are prevalent in developed countries, where there is enormous market potential for any novel alternative treatments discovered. In this review, we provide a brief outlook on the mechanisms of action of these biotherapies, and summarize their roles in human and veterinary medicine.

Keywords Helminth, Maggot, Leech, Product, Parasite

Introduction

Biotherapy, also known as biological therapy or biological response modification, is a traditional-turned-modern concept of using living cells, tissues, or whole organism as a prophylactic/therapeutic measure to modulate immunological responses. Animal-assisted therapies encompassing phages, helminths, arthropods, annelids, and vertebrates are the disciples of biotherapy; such organisms have been shown to alter the pathways of several incurable diseases. The prime motive of such therapies is to mask the pathogenicity of the disease and regulate its pathway without causing undue pressure on the host's immune system [1]. Advances in molecular biology and genetic engineering have driven the development and use of biologicals, such as recombinant proteins, cytokines, and chimeric monoclonal antibodies, to alter the courses of diseases in patients with erratic responses to conventional treatment alone [1]. Such biologicals in synergy with chemotherapeutics have been used extensively in the treatment and management of immune-mediated disorders and, to some extent, in wound care [2]. Parasites, as unwelcome tenants to their hosts, are an important biological source. At low levels, they are beneficial boosters of the immune system [3], producing excretions and secretions (ES) that can aid in wound healing [4]. The spectrum and mode of action of parasitic diseases are nearly identical in humans and animals; this similarity can serve as an early sign of environmental hazard, indicating the need for public health intervention [5]. In this review, we focused on the beneficial interactions of parasites with their human and animal hosts, exploring the use of parasites as a therapeutic source in human and veterinary medicine (Fig. 1). With

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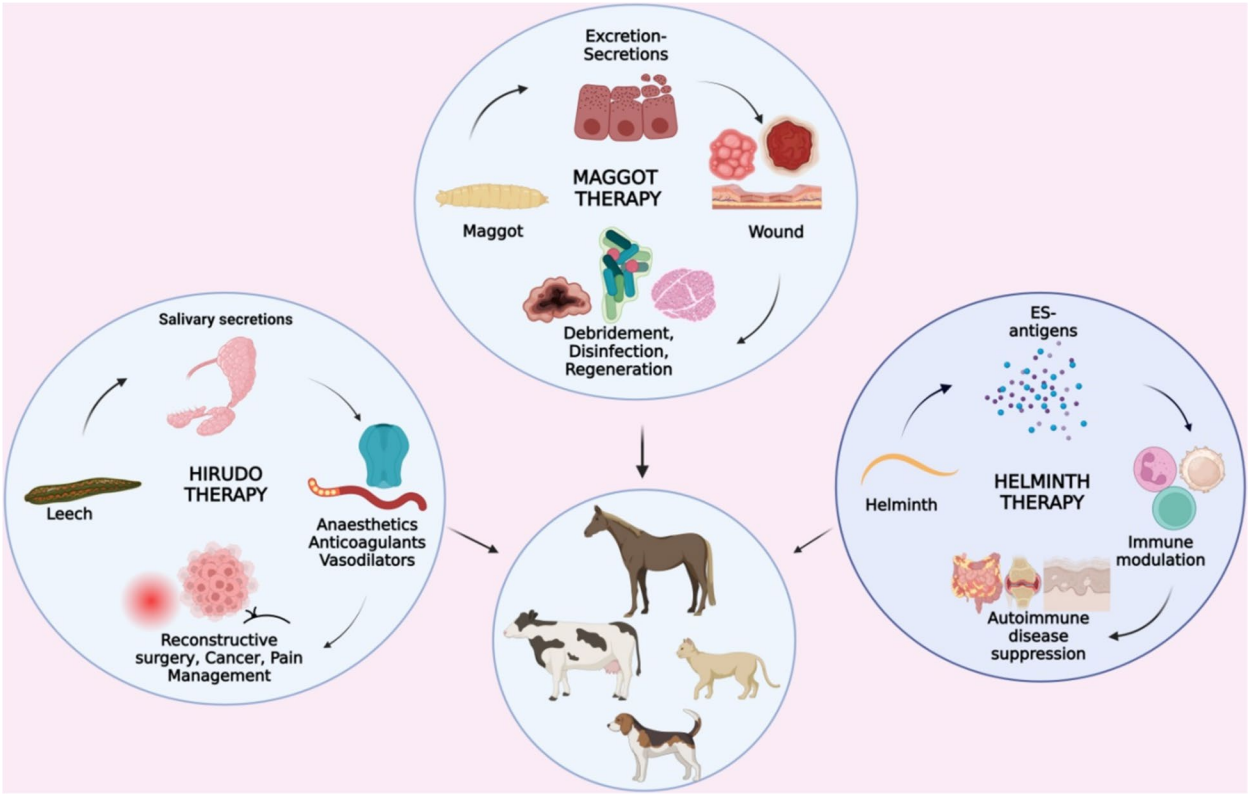

Fig. 1 Pictorial representation of various therapies that utilize parasites to cure livestock diseases

the increasing problem of drug resistance, alternative traditional therapies are essential to address various ailments. The identification and application of novel candidate ES in animal and human trials may provide insights into their roles in mitigating autoimmune diseases and allergies, and managing chronic wounds.

Helminth therapy

Humans have complex relationship with many helminth parasites, namely the nemathelminths (roundworms) and platyhelminths (cestodes and trematodes), which have effectively co-adapted with their respective hosts by regulating the host's immune response. Interestingly, among human hosts, those of pre-reproductive age are the perfect targets for helminths. Unfortunately, in the absence of clinical manifestations, young hosts are not often administered anti-helminthic treatments, thus typically leading prolonged chronic infection. Over time such close-knit conditions tend to lead to parasitic tampering with the host immune system in one of two ways: (1) selective pressure on genes responsible for regulating cytokine expression levels [6]; or (2) evasion of the host's immune system through a series of immunomodulatory mechanisms [3]. During this process, such parasites inadvertently suppress autoimmune diseases. This protective phenomenon is supported by a number of hypotheses (e.g., hygiene hypothesis, biodiversity hypothesis, and biome depletion theory) that all point to an inverse relationship between the parasitic infection and the occurrence of a chronic inflammatory disease in the host [7]. However, synergistic advancements in technologies and medical facilities have reduced the colonization of parasitic populations, especially in developed nations, and have coincided with increased incidences of allergic and autoimmune infections. Such illnesses can be prevented by helminthic manipulation of the host immune system.

Host immune modulation by helminths

The immune system comprises a variety of cells, organs, and factors that carry out the surveillance and elimination of pathogenic organisms. The entry of pathogens is recognized by Toll-like receptors (TLRs), a type of pattern recognition receptors (PRRs) that identify both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [8]. TLRs expressed by various cells, including granulocytes, agranulocytes, macrophages, mast cells, natural killer cells, dendritic cells, and fibroblasts, generate effective innate immune responses [9].

Different cell types express different groups of TLRs, depending on the structure, composition, and immunogenic response of the pathogen, and the recognition of PAMPs and DAMPs [10]. TLR activation leads to the recruitment of certain cellular moieties that protect and repair the damaged tissues by releasing inflammatory cytokines. However, uncontrolled expression of TLRs can lead to upregulation of the pro-inflammatory cytokines (e.g., interleukin-6 (IL-6), IL-12, IL-17, tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ)), chemokines, and type 1 IFN, which is responsible for the progression of autoimmune diseases [8]. This overexpression can be curbed by helminths. An experiment conducted by Pineda and co-workers [11] demonstrated that ES-62, a glycoprotein secreted by the helminth *Acanthocheilonema vitae*, causes segregation and suppressed signalling of TLRs and IL-33, a proallergic alarmin cytokine. In another study, a recombinant F12 secretory molecule of the liver fluke *Fasciola hepatica* was found to inhibit pro-inflammatory cytokine production through suppression of macrophage activation mediated by TLRs (2, 4, 5 and 8) [12]. Similarly, the human blood fluke *Schistosoma mansoni* releases cathepsin B1, which inactivates MyD88-independent pathways to suppress the production of TLR3 and TLR4, thus modulating the T helper cell 2 (Th2) response [13].

Generally, Th1, Th17, and Th2 responses, as key components of $CD4^+$ T cell subsets, provide protection against intracellular pathogens (bacteria and protozoa), autoimmunity, and extracellular pathogens (helminths and ectoparasites), respectively. Individuals with autoimmune infections show an elevated Th1/Th17 immune response, with an upregulation of pro-inflammatory cytokines [14]. These cytokines assist neutrophils and macrophages in neutralizing their targets; however, an exaggerated Th1/Th17 response may be responsible for the destruction of healthy tissue [15]. Such responses can be modulated in individuals harbouring a helminth infection. Helminths instigate the Th2-type immune response, whereby producing IL-4, IL-5, IL-9, IL-10 and IL-13 [14]. Two of these cytokines, IL-4 and IL-5, mediate the growth and differentiation of plasma B cells, promote eosinophilic infiltration, regulates the production of immunoglobulin E (IgE), tuft cells, and mucus, and impact mastocytosis; however an uncontrolled response can pave the way for allergic disorders [6]. IL-10, a potent anti-inflammatory cytokine, along with the suppressive activities of regulatory T cells (Tregs) and B cells (Bregs), activated macrophages, and dendritic cells, help to curb activated Th1 and Th2 immune responses [16]. In a mouse model experiment with *Heligmosomoides polygyrus*, the $IL\text{-}10^+ FOXP3^+$ Treg population was maintained by $IL\text{-}10^+$ Bregs, suggesting that the role of Bregs is not overshadowed by subsets of T-cells [17] (Fig. 2).

Therapeutic interventions with helminths

Humans

Used as a therapeutic tool for immunity-related ailments, including type 1 diabetes, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, asthma, eczema, and systemic lupus erythematosus, helminth-based treatments may involve specific ES components or the whole parasite. Scientists worldwide have aimed to enhance helminth-derived products to cure or alleviate the suffering of immunocompromised patients [6] through experiments on animal models [18–20] and human clinical trials [21–23]. Unsurprisingly, while helminths have been widely tested in animal model experiments (Tables 1 and 2), few have progressed to human clinical trials. This is because certain laboratory helminths, such as *Nippostrongylus brasiliensis* and *H. polygyrus,* are known for migrating to sanctuary sites and causing chronic pulmonary disorders in animal models, casting doubt on their safety for clinical settings [24]. Notably, three nematodes and one cestode have been widely exploited in clinical trials and used by several self-medicating individuals, who have provided feedback to physicians involved in practising helminth therapy and their suppliers [25]. The eggs of the swine whipworm *Trichuris suis* have been used primarily for the treatment of inflammatory bowel diseases (e.g., ulcerative colitis and Crohn's disease) [22], while the larvae of the human hookworm *Necator americanus* have been shown to ameliorate the symptoms induced by inflammatory bowel disease [26] and allergic rhinitis [27] in human clinical trials. The human whipworm *Trichuris trichiura* exerts immunomodulatory effects that have been studied in autism, allergies, and asthma [28], while cysticercoids of the rat tapeworm *Hymenolepis diminuta* have been utilized to treat neurological disorders including multiple sclerosis [29]. The success of such trials has been challenged by certain drawbacks related to the parasites themselves, including pathogenic potential, aberrant migration to sanctuary sites [30], re-infection, increased host susceptibility to bacterial infections [31], compromised immunoregulation in response to cancerous agents [6], and unpremeditated transmission of other pathogens [25]. Compared with *T. trichiura*, *H. diminuta,* and *N. americanus*, production of *T. suis* is more expensive, owing to its requirements for replication in pigs, isolation of infected ova under specific laboratory conditions, and the additional cost of recurrent re-colonization every 2 weeks [28].

While helminthiasis remains a problem in less economically developed countries, autoimmune diseases are

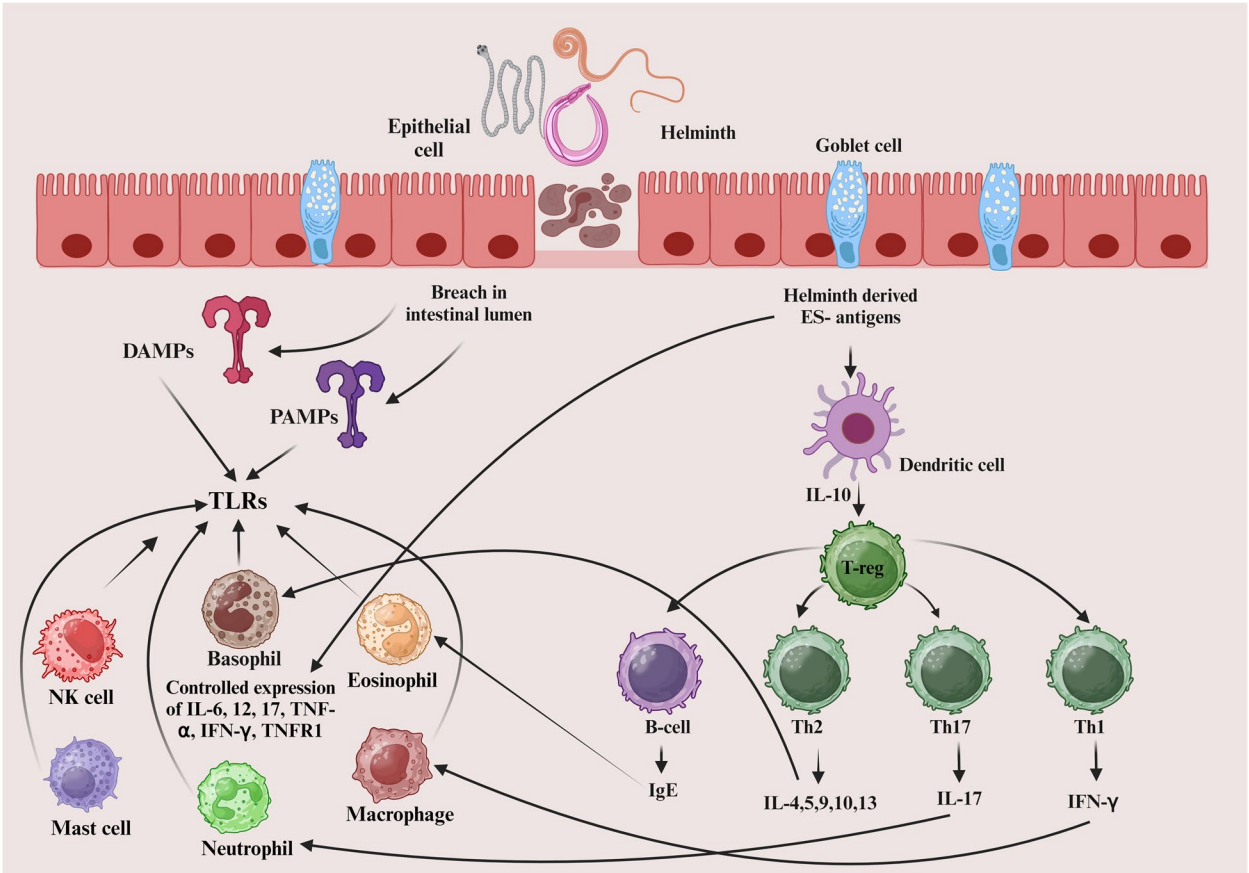

Fig. 2 Host immune modulation in response to helminth infection

on the rise in more developed countries. In places with adequate funding and infrastructure, large numbers of experiments have been conducted using helminths in murine models. Similar studies in insect models would be beneficial. *Drosophila melanogaster* is an ideal insect model for such studies because of its short life cycle, good biotic potential, relatively simple body structure, amenability to genetic manipulations, and exemption from ethical approval. The cellular immune response of the fruit fly is mediated by three categories of haemocytes, namely plasmocytes (phagocytotic moieties), crystal cells (melanizing foreign antigens and wound repair), and lamellocytes (pathogen encapsulation) [63]. These insects also have antimicrobial peptides for opsonizing and phagocytosing microbes. Recently, axenic nematodes such as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* have been used to infect insects, expanding our knowledge of host-parasite relationships [64]. Apart from insects, zebrafish and their transparent counterparts are being considered as a potential model to study the immune response to parasites such as *H. polygyrus* [64, 65].

Because many patients are disgusted by the idea of being treated with a live parasite, efforts are being made to identify and isolate helminthic molecules with immunomodulatory potential [66]. Several such molecules have been documented [15, 37, 42, 48, 55], but none have been administered in human trials [6].

Animals

The use of helminths to treat autoimmune and allergic ailments in animals is still in its infancy. Currently, it is unclear whether helminths can be used to treat autoimmune diseases in animals as effectively as in their human counterparts. There is evidence that the removal of helminths may disturb the equine bacterial microbiota, paving the way for inflammatory conditions [67]. Commensal bacteria form a part of the animal gut environment at birth, and their interactions with helminths can change the microbiota composition, thereby influencing the courses of several diseases [68]. Furthermore, these

Table 1 Studies of human autoimmune diseases using helminth parasites or by-products in animal models

Autoimmune Disease	Helminth parasites / their by-products	Model	Outcomes	References
Type 1 Diabetes (T1D)	*Schistosoma mansoni* soluble egg/worm antigen	Non-obese Diabetic (NOD) mice	Effective when administered at 4 weeks of age. Causes decrease interleukin-12 (IL-12) and increase IL-10 production	[32]
	Trichinella spiralis	NOD mice	Provides protection mainly by a prejudiced Th2 response coupled with an IL-10 response	[33]
	Heligmosomoides polygyrus	Streptozotocin (STZ)-induced T1D mouse model	Protective effects against hyperglycemia and decrease in pancreatic islet size through Th2 independent mechanisms	[34]
	Fasciola hepatica excretory secretory antigen	NOD mice	Promotes interferon-γ (IFN-γ) suppression and modulates macrophage activity	[35]
	Litomosoides sigmodontis antigen	NOD mice	Antigen administration with intranasal pro-insulin dose increases Treg cell frequency and decreases pancreatic islet inflammation	[36]
	Brugia malayi antigen	STZ induced T1D mouse model	rBmALT-2 decreases tumour necrosis factor-α (TNF-α) and IFN-γ levels, and increases IL-4, IL-5, and IL-10 production by splenocytes	[18]
	Schistosoma japonicum antigen (Cystatin and fructose-1,6-bisphosphate aldolase)	NOD mice	Disease amelioration by decreasing IFN-γ and increasing Th2 cytokines, Tregs, IL-10 and TGF-β	[37]
Rheumatoid arthritis (RA)	*Nippostrongylus brasiliensis*	Murphy Roths Large (MRL) *lpr* mice	Parasitic infections decrease the incidence and severity of arthritis. Increased serum concentration of IL-4 and IgG1 are noted	[38]
	Fasciola gigantica somatic antigen (Fg-Ag) and *Gigantocotyle explanatum* (Ge-Ag) somatic antigen	Collagen induced arthritis (CIA) rats	Therapeutically, Ge-Ag is more effective than Fg-Ag, demonstrated by reducted serum TNF-α and IFN-γ levels and increased serum IL-4 and IL-10 levels	[39]
	Schistosoma mansoni	IL-1 receptor antagonist (IL-1Ra)-deficient mice	Mitigates disease impact by decreasing IL-17 and TNF-α while increasing IL-4 and IL-10 splenic responses	[40]
	Acanthocheilonema viteae ES-62	CIA mice	Downregulation of IL-1β and decrease in inflammasome activity at the site of infection	[15, 41];
	Schistosoma japonicum peptide SJMHE1	DBA/1 J mice	Peptides with minimal side effects effectively suppress collagen-induced arthritis symptoms by downregulating IFN-γ, TNF-α, IL–6, IL-17, and IL-22, and upregulating IL-10 and $CD4^+$ Tregs	[42]
	Clonorchis sinensis metacercariae	CIA mice	Aggravated arthritis due to increased neutrophils and monocytes while decreased B cells and $CD4^+$ T cells count	[19]
	Trichinella spiralis MES	CIA mice	ES products inhibit M1 macrophage polarization and proinflammatory cytokines in CIA mice, thereby reducing bone destruction and inhibits osteoclastogenesis in infected joints	[43]

Table 1 (continued)

Autoimmune Disease	Helminth parasites / their by-products	Model	Outcomes	References
Multiple sclerosis (MS)	*Schistosoma mansoni* ova	Experimental Autoimmune Encephalomyelitis (EAE) Mice	Immune response polarization; decrease in IFN-r, TNF-α, IL-12, and increase in IL-4, IL-10, and TGF-β; Th2 response was observed	[44]
	Schistosoma japonicum Soluble Egg Antigen (SEA)	EAE Mice	Ameliorates disease by decreasing IFN-γ and increasing IL-4 production in the CNS and spleen	[45]
	Taenia crassiceps	EAE Mice	Decreases IL-17 and TNF-α production and increases IL-4 and IL-10 production	[46]
	Toxascaris leonina recombinant galectin (rTl-gal)	EAE Mice	Prevents EAE remission by markedly increasing CD45R/B220^{+}B cell numbers and decreasing TNF-α and IFN-γ	[47]
	Fasciola hepatica FhHDM-1 peptide	NOD/Lt mice	Ameliorates disease by reducing the secretion of pro-inflammatory cytokines such as TNF and IL-6	[48]
Inflammatory Bowel Disease (IBD)	*Schistosoma mansoni* egg antigen	Dextran sodium sulfate (DSS) induced colitis in mice	It reduces disease effect by increasing FoxP3^{+}T regulatory cells and Th2 cytokine production	[49]
	Heligmosomoides polygyrus ES 55 kDa antigen	DSS induced colitis in BALB/c mice	Antigen treatment modulates IL-10 production, thereby reducing inflammation caused by DSS-induced colitis	[50]
	Hymenolepis diminuta	DNBS induced colitis in BALB/c mice	The helminth-infected mice showed increased IL-4 and IL-10 production	[51]
	Trichinella spiralis Cystatin	Trinitrobenzene sulfonic acid (TNBS) induced colitis in BALB/c mice	Protection was achieved via an induced Th2-type response	[52]
	Hymenolepis diminuta	Dinitrobenzene sulfonic acid (DNBS) induced colitis in mice	A skewed Th2 response was observed	[53]
	Schistosoma japonicum peptide SJMHE1	C57BL/6 mice	The peptide demonstrates protective effects against both acute and chronic colitis induced by DSS	[20]
	Echinococcus granulosus sensu strict and Antigen B	DSS infected BALB/c mice	A reduction in clinical symptoms was achieved owing to increased F4/80^{+} CD206^{+} and decreased F4/80^{+} CD11c^{+} in the intestine	[54]
	Clonorchis sinensis cysteine proteases (r*Cs*CP and *Cs*CA)	DSS induced colitis in mice	Ameliorates colitis by upregulating IL-4, IL-10, and IL-13 and downregulating IL-12b, IL-23r, IL-7, and IL-17A production	[55]

bacteria regulate type 2 immune responses via Tregs [69], inhibiting the actions of pro-inflammatory cytokines. Such gut microbiota-related activities can reduce the risks of allergic and inflammatory conditions [70, 71]. *Toxocara canis* and *Ancylostoma caninum* ES antigens have been shown to regulate the canine immune system by modulating $Foxp3^{high}$ T cells. This results in an increase in $CD8^{+}$IL-10^{+}T cells, ultimately preventing polyclonal T-cell proliferation and dendritic cell maturation [72] and leading to a skewed Th2 response. Such excretory products are also known to improve wound healing in pets [73]. Similarly, thioredoxin peroxidase present in the ES antigens of *Cysticercus cellulosae* was found to elevate levels of $CD4^{+}$ $CD25^{+}$ $Foxp3^{+}$ Tregs in the piglets, leading to an upregulation of IL-4 and IL-10 levels and consequent downregulation of the Th1/Th17 response [74]. Such experiments shed light on the mechanism of parasitic evasion, but also reveal possibilities for using ES antigens to suppress the expression of allergic and autoimmune diseases in pets and farm animals. However, it is up to farm owners and pet parents to volunteer for such treatments.

Maggot debridement therapy (MDT)

MDT, also known as maggot therapy, larval therapy, larval debridement therapy, biosurgery, biodebridement and wound myiasis is a method entailing the application of sterile medical-grade live larvae of *Lucilia* (*Phaenicia*) *sericata* (green bottle fly) onto a wound in a controlled environment. Briefly, MDT is a form of aseptically controlled therapeutic wound myiasis [75]. In nature, flies lay eggs on carrion and wounds, where they hatch, feed, and moult to form three larval stages (instar) known as maggots [76]. Metamorphosis (pupae and adult) also occurs in the wound environment. This type of necrophagous behaviour has long been exploited in the treatment of chronic and necrotic wounds [75, 77].

First instar maggots are very active feeders [78]. Mechanically, they work their magic via spicules that scrape away cellular debris or necrotic tissue. They also have modified mandibles called "mouth hooks" that are used for locomotion and raking the unhealthy tissues [79], but are not involved in tissue consumption [80]. Effective debridement also helps to eliminate the foul smell associated with these wounds. Although different methods of debridement are currently available, including surgical, autolytic (hydrogels and hydrocolloids), enzymatic (proteolytic enzymes), mechanical (wet, dry, or impregnated gauge), and biological (larval) [81], biological debridement is the fastest, and features almost negligible live tissue involvement [82].

Role of larval ES

While crawling over wounds, maggots secrete and excrete extracorporeal digestive enzymes and alimentary secretions that penetrate and liquefy the necrotic tissue, enabling them to ingest it [83]. The digestive secretions of maggots encompass a diverse array of components. These include enzymatic substances, such as trypsin and chymotrypsin (specifically LCTa and LCTb), leucin aminopeptidase, and carboxypeptidase A and B. The secretions also contain collagenases, glutathione, and sulfhydryl radicals. Serine proteases are present, along with an aspartyl proteinase and an exopeptidase resembling matrix metalloproteinases. The composition is further complemented by calcium carbonate, urea, and allantoin [84–86]. Larval trypsin and chymotrypsin are highly resistant to degradation by endogenous wound inhibitors [87]. The actions of matrix metalloproteases on the wound release ammonia, which increases the wound pH and activates proteases that ultimately degrade components of the extracellular matrix, such as collagen types I and III, fibronectin, and laminin [88, 89].

The beneficial effects of larval ES on chronic wounds are exerted through antimicrobial activity, haemostasis, angiogenesis, cell proliferation, tissue granulation, growth factor-mediated promotion of extracellular matrix remodelling, and increased levels of anti-inflammatory cytokines [80, 85].

Natural wound healing begins with haemostasis. Platelet plugs, in association with the coagulation cascade, form an insoluble mesh of fibrin fibres (clot) [90], preventing further bleeding and providing a reservoir of growth factors for cell migration [91]. In acute wounds, the clot is further degraded via the activation of the serine protease urokinase plasminogen activator [92]. However, chronic wounds contain high levels of plasminogen activator inhibitor-150, which impairs fibrinolysis and thus prevents wound closure [93]. Maggot secretions enhance plasmin formation, induce fibrinolysis, break down the fibrin slough that accumulates in chronic wounds, and promote the neovascularization and growth of granulation tissue, thus helping the wound to heal [93]. An in vitro study conducted by Kahl et al. [94] showed that larval serine proteases could induce clotting in human plasma and whole blood by surpassing fibrinolysis activity. In another study, it was observed that *L. sericata* Jonah chymotrypsin, a recombinant protein produced in *Escherichia coli*, reduced the clotting time in human plasma, along with effective digestion of fibronectin, laminin, and collagen IV [95].

Haemostasis is followed by an inflammatory phase. The complement system is an important component of the host immune system, which can be activated through classical, alternative, or lectin pathways [96].

Table 2 Studies of human allergic diseases using helminth parasites or by-products in animal models

Allergic Disease	Helminth parasites/their by-products	Model	Outcomes	References
Asthma	*Schistosoma mansoni* eggs and cercariae	Ovalbumin (OVA)-induced experimental asthma in BALB/c	Decreased IL-4, IL-5, IgE levels, and Th2 production and increased $CD4^+CD25^+Foxp3^+$T cell production	[56]
	Acanthocheilonema viteae ES-62 antigen and Small Molecule Analogs (SMAs) 11a and 12b	OVA-induced mice model (C57BL/6J)	A suppressed Th17/Th2 mediated and neutrophilic airway allergic inflammatory responses were recorded	[57]
	Heligmosomoides polygyrus Alarmin Release Inhibitor (HpARI)	BALB/cOlaHsd, C57BL/6JOlaHsd, IL-13-eGFP and ST2-deficient (BALB/c) mice models	HpARI revoked IL-33, by reducing eosinophilic responses to *Alternaria* allergen administration	[58]
	Trichinella spiralis adult worm extract (*Ts*-AE) and muscle larvae extract (*Ts*-MLE)	Female BALB/c mice: OVA-induced asthma mouse model	*Ts*-AE decreased OVA-specific IgE, eosinophil infiltration, IL-4 and increased IL-10 and TGF-β	[59]
Systemic lupus erythematosus (SLE)	*Acanthocheilonema viteae* ES-62	*gld*.apoE$^{-/-}$ mouse model	Infected mice showed low levels of macrophages and anti-nuclear autoantibody, which reduced the percentage of accelerated atherosclerosis by 60%	[60]
	Hymenolepis microstoma	NZBWF1 mice	A lower count of activated lymphocytes and higher levels of regulatory T cells in the lymphoid organs were observed	[61]
Eczema	*Heligmosomoides bakeri*	Dibutyl phthalate fluorescein isothiocynate (DBP-FITC) sensitized C57BL/6 mice	Suppressed cytokine production, modulating allergic responses	[62]

Inappropriate activation of the complement system can prolong the inflammatory phase and prevent wound healing. Cazander et al. [97] documented that maggot ES can alter the course of complement system by degrading the complement components C3 and C4. During inflammation, neutrophils defend the immune system by phagocytosing invading pathogens. This causes increased production of hydrogen peroxide, superoxide, and hydroxyl radical, and results in an extended inflammatory period [98]. However, a detailed study designed to extrapolate the effect of maggot ES on opsonized zymogen-stimulated and unstimulated neutrophils found that there was a significant decrease in the release of superoxide and myeloperoxidase, but only in the stimulated neutrophils [99]. Monocytes are also associated with neutrophils during the innate immune response. Once infection occurs, they differentiate into pro-inflammatory and anti-inflammatory, or pro-angiogenic, macrophages. Under chronic infection, pro-inflammatory macrophages produce TNF-α, IL-1α, IL-1ß, IL-12, and macrophage migration inhibitory factor, damaging the extracellular matrix, causing the inactivation of growth factors, and preventing proper wound healing [100, 101]. Anti-inflammatory macrophages produce IL-10, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF), which promote cell proliferation and neo-vascularization [100]. Consistently, Li et al. [102] and Tellez et al. [103] demonstrated that larval ES negatively impacted pro-inflammatory cytokine levels while upregulating the expression of anti-inflammatory cytokines through a cAMP-mediated process. In another study, blowfly larval immunosuppressive protein extracted from *Lucilia cuprina* larvae downregulated the mRNA expression levels of IFN-γ, IL-4, IL-10, and IL-13, reduced mitogen-induced lymphocyte proliferation, and ameliorated the infection by upregulating the expression levels of anti-inflammatory cytokines TNF-α and transforming growth factor β (TGF-β) [104].

The beginning of new tissue formation triggers anti-inflammatory macrophages to produce pro-angiogenic factors (e.g., VEGF and bFGF), which provide oxygen and are required to nourish the cellular components involved in wound healing [105]. Bexfield et al. [106] found that pro-angiogenic factors (e.g., l-histidine, 3-guanidinopropionic acid, and l-valinol in the ES of maggots promote wound healing through angiogenesis and strengthening vascular endothelial migration, but have no effects on fibroblasts. Sun et al. [107] reported increased expression of VEGF receptor 2 and upregulated production of endothelial cells after the application of larval ES on a diabetic foot ulcer in patients. In a mouse experiment, fatty acid extracts derived from dried *L. sericata* larvae were applied to cutaneous wounds, and their activities were recorded on days 3, 7, and 10 post wound creation. The extracts promoted wound healing, particularly during the inflammatory and granulation formation phases. They upregulated VEGFA expression during the inflammatory phase, enhancing wound contraction in later phases [108]. A recent study on the activity of angiogenesis-related microRNAs (miRNAs) associated with larval ES-treated human umbilical vein endothelial cells used to counter diabetic foot ulcer found upregulated expression of miR18a/19a transcription factors and downregulated expression of TSP-1 [109].

The migration of epidermal cells into the wound bed is a key step in wound healing. The phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt) pathway is an important regulator of cell migration. Changes in certain growth factors trigger PI3K activation, such as loss of phosphatase and tensin homolog (PTEN), or increased expression of epidermal growth factor receptor (EGFR). Once PI3K is activated, it increases VEGF production. VEGF binds to receptors on endothelial cells to activate the RAS and PI3K pathways and promote angiogenesis and vascular permeability [109, 110].

Extracellular matrix proliferation begins with the organization of the cellular components of fibroblasts: fibronectin, elastin, and collagen (type III) [111]. Fibroblasts play a central role in granulation tissue formation, along with macrophages, blood vessels, granulocytes, chemokines, and cytokines. The extracellular matrix components displace fibrin clots with cells that promote re-epithelialization, such as keratinocytes [112]. As proliferation progresses, fibroblasts differentiate into myofibroblasts, thus entering the remodelling phase. Here, type I collagen replaces type III to provide strength [113]. Apoptosis of inflammatory cells also occurs, causing a reduction in cellular content with a regression in blood vasculature. Finally, a scar is formed as wound repair concludes [114].

Medicinal maggots and wound therapies

Production and rearing of medicinal maggots

Medicinal maggot production requires the availability of laboratory facilities appropriate for research or commercial purposes. Ideally, an insectary for rearing and maintenance of colonies requires a constant temperature of 25 ˚C, a relative humidity of 40% to 60%, and 12 h of light per day to ensure uninterrupted egg production, without any possibility of the larvae entering diapause during pupariation [115]. Laboratory-reared flies are fed a special diet that includes carbohydrates (honey mixed with water and food enriched with sugar) and protein (brewer's yeast, dried milk, agar powder, and liver in pieces) to support the maturation of ovaries and oviposition [116]. Subsequently, the eggs are disinfected with 0.5% sodium

hypochlorite or 3% Lysol®, and the hatched maggots are reared on sterile media. Successful disinfection testing is followed by quality control assurance, storage, and scheduled production activities [117]. Presently, medicinal maggots are commercially supplied by BioMonde, Monarch Labs, and the International Biotherapy Society.

MDT in human and veterinary medicine

In humans, MDT has been used to treat a plethora of wound infections involving necrotic, open, non-healing chronic, soft tissue, gangrenous, and deep cavity wounds [118]. Apart from chronic wounds, acute wounds with an immediate need for debridement can also be treated with this therapy. MDT is often viewed as a last resort by patients who have tried every other available treatment before succumbing to the pain and mental trauma of their unhealed wounds. MDT is a good choice for ulcers, except for corneal ulcers, where the wriggling movements of the maggots can cause scarring and damage to delicate eye tissues [119]. Other contraindications include advanced life-threatening infections, sterile cavities, ailments involving the bone and tendons, and bleeding complications [120]. Given the tendency to value humans over animals, MDT has not been applied with the same enthusiasm in animals; as such, the indications and benefits of this therapy remain unknown to many veterinarians. There are very few documented reports of MDT in veterinary medicine, with most cases involving large animals, especially horses; only recently has this therapy been used for domestic pets [121] (Table 3). However, the actural number of treated cases remains unknown. Furthermore, the success rate of treatment in veterinary medicine is comparatively low, likely because the probability of animals removing their dressings is high. Other limitations of MDT in animals include the non-availability of medicinal maggots, timeliness of treatment, and cost efficiency [122] (Fig. 3).

Substitution for the green bottle fly

L. sericata, the green bottle fly known for outstanding safety in MDT, is not available in every geographical region. Therefore, it is important to identify local fly species that can serve as an alternative in larval therapy (Table 4). Standards for the selection of medicinal fly species include the following: manageable life cycle, ease of breeding and maintenance, strictly necrophagous feeding, and no intra-specific competition [140]. Flies that tend to exhibit obligatory myiasis should be avoided at all costs. Certain fly species (e.g., *Lucilia exima*) switch over an opportunistic parasitic mode under certain conditions [141], whereas others (e.g., *Chrysomia albiceps*), being necrophagous, switch to cannibalism under intense starvation [142].

Before conducting the clinical trials, the mode of action and safety of the flies are tested in laboratory animals. Trials using safety-tested flies are conducted in four phases, with each phase involving increased participation of volunteers (patients with chronic wounds) and strictly following ethical guidelines and national norms. Satisfactory maggots are then commercially bred from reliable sources and delivered to hospitals or clinicians [140].

Hirudotherapy

Hirudotherapy or medicinal leech therapy (MLT) is a painless procedure that utilizes the salivary secretions of medicinal leeches by allowed to feed on a site of infection. While there are several species of medicinal leeches, most commonly used species of the genus *Hirudo* include *H. medicinalis*, *H. asiatica*, *H. granulose*, *H. verbena* and *H. orientalis*. Leech salivary glands harbour several bioactive substances that exert the following pharmacological effects: anticoagulation (hirudin, destabilase, lefaxin, gelin, new leech protein-1, ghilanten) [161–164]; anaesthetization; vasodilation (acetylcholine); anti-inflammation (antistasin, eglins, hirustasin, and carboxypeptidase inhibition) [164–166]; platelet inhibition (saratin, calin, decorsin, apyrase) [161, 165]; antimicrobial activity (destabilase, theromacin, chloramphenicol, peptide B) [165, 167]; thrombin regulatory functions (gelin, hirudin); extracellular matrix degradation (collagenase, hyaluronidase); and analgesia (guamerin, piguamerin, bdellins) [161]. Thess substances have been observed to re-establish the vascular permeability of organ systems, boost the immune response, decrease blood pressure, remove hypoxia, and quash microcirculatory problems [168].

Hirudotherapy in human and veterinary medicine

Leeches have been used to cure diseases since ancient times, with the first recorded document dating back to 1500 B.C. [169]. Several reports have provided insight into the mechanisms of action and benefits of leech therapy for treating complicated medical conditions [164, 170]. Hirudotherapy is most commonly used to treat necrotic flaps, post-phlebitic syndrome, haematomas, and polycythaemia in plastic and reconstructive surgeries [171, 172]. Surgeries pertaining to the amputation and reattachment of tissues involve anastomoses of tiny blood vessels, with the inevitable complication of blood accumulation (venous congestion), which can lead to thrombosis and ultimately tissue necrosis [173]. Leeches applied to the area concerned actively drain excess blood, and the anticoagulant effect of the leech saliva promotes the oozing of additional blood even after the leeches have been removed [173], helping to alleviate venous congestion in affected patients. Remarkable effects of leech

therapy against tumor metastasis and the associated pain have also been noted [174]. Specifically, ghilanten, a potent anticoagulant present in the saliva of *Haementeria ghilianii*, possesses antimetastatic activity against cancerous cells of melanomas, and lung, breast, and prostate tumors [169] (Table 5).

Leech therapy can sometimes lead to certain complications, the most common being infection. The symbiotic association between leeches and the bacterium *Aeromonas hydrophila* can cause secondary bacterial infections in patients undergoing leech therapy, necessitating antibiotic treatment [194]. Leech therapy is contraindicated in patients with anaemia, excessive bleeding, haemophilia, hypotension, sepsis, allergic reactions, and cutaneous conditions (e.g., necrotic ulcers, pruritus, cutaneous pseudolymphoma, and allergic dermatitis) [172, 195]. Additionally, pregnant and lactating females should not undergo such treatment [185].

In veterinary medicine, the indications and contraindications for leech therapy are on par with human medicine (Table 5). In animals (horses, dogs, and cats), it has mainly been used to salvage venous congestion [185]. Some of the common indications for leech therapy include neuritis, myositis, ataxia, discopathies, caudal equina syndrome, post-surgical scars, and spinal injuries [196]. Recently, the focus has shifted towards the treatment of animal protozoal diseases. *Hirudo* extract antigens (HEA) identified by Al-Sayed and co-workers [197] were evaluated for their protective efficacy against murine eimeriosis caused by *Eimeria papillata* [197]. The pathogenic effects of *Eimeria* considerably increase the production of nitric oxide and malondialdehyde, resulting in oxidative stress [198], while reducing antioxidants (e.g., superoxide dismutase, glutathione, and catalase), leading to increased cellular damage, lipid peroxidation, and apoptosis [199]. These effects are responsible for causing inflammation of intestinal cells and hampering normal digestive mechanisms. Infected mice injected with HEA showed downregulated expression of IFN-γ, TNF-α, and IL1-ß, and an upregulated expression of IL-10 [197]. An upregulated IL-10 response promotes goblet cell formation and mucus production, thereby forming a protective shield against such infections [200]. In addition to HEA, Eglin C has shown promising therapeutic effects in curbing *E. papillata*-induced coccidiosis [201]. The authors of these studies suggested that such alternative therapies can reduce clinician overdependence on anticoccidial treatments.

Conclusion and future perspectives

Parasites, considered both friends and foes, are the future of many alternative therapies. Helminths proficiently manipulate the immune system, inadvertently benefiting the host to a certain extent. The plethora of complex immunomodulatory substances (ES antigens) released by helminths poses a challenge for human trials, although studies in murine models have shown promising results. Even more daunting is the need to formulate

Table 3 Case studies of MDT in animals

Species	Indications	References
Equine	Canker, Quittor, Navicular bursa sepsis, Septic arthritis	[123]
	Non healing foot ulcer, Chronic digital interphalangeal joint sepsis, Coffin bone rotation	[124]
	Suppurative panniculitis	[125]
	Osteomyelitis	[126]
	Septic navicular bursitis, Chronic laminitis	[127]
	Supraspinous bursitis	[128]
	Puncture wounds in navicular bursa	[129]
	Laceration of limbs, Fistulous withers, Linea alba dehiscence	[130]
	Abdominal wound	[131]
	Snake bite necrotic wound	[132]
	Sarcoid lesions	[133]
Ovine	Interdigital skin inflammation (foot rot and foot scald)	[134]
	Necrotic wounds in skin	[135]
Canine	Pressure ulcer, gunshot wound	[121]
	Deep tissue necrotic wounds under footpads	[136]
	Necrotic wound	[137]
	Traumatic wounds in thoracic limbs	[138]
Feline	Fibrosarcoma, multiple bite wounds	[121]
	Post-operative infected wound	[139]

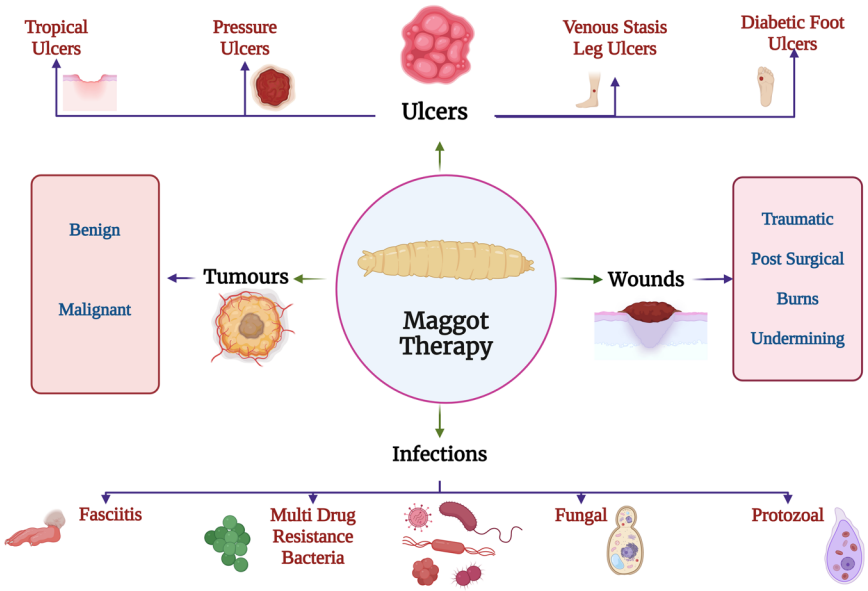

Fig. 3 Therapeutic uses of maggots for different ailments

Table 4 Candidate fly species and their secretory products and properties utilized in maggot-based therapies

Species	Target Peptide / Excretions Secretions (ES)	Properties	References
Calliphora vicina	Alloferon 1 and 2	Antibacterial action against antibiotic resistant *E. coli*, *S. aureus*, *A. baumannii* biofilms; antifungal activity against *Candida albicans*; antiprotozoal activity against Cutaneous Leishmaniosis	[143–146]
Chrysomia megacephala	ES	Antibacterial activity, Promotes egg disinfection efficiency	[147]
Cochliomyia macellaria	ES	Antibacterial activity against *Staphylococcus aureus*	[148]
Lucilia eximia	Lucilin	Antibacterial action against Gram negative bacteria, potent immunomodulator decreasing production of TNF-α	[149]
Lucilia sericata	Lucifensin, Lucimycin	Antibacterial action against biofilm, antifungal activity, antiprotozoal activity against Cutaneous Leishmaniosis, promotes fibroblast migration and angiogenesis	[106, 146, 150, 151]
Lucilia cuprina	Lucifensin II	Antibacterial action against multidrug resistant *Staphylococcus aureus*	[152, 153]
	ES	Virucidal properties against Rift Valley Fever and Coxsackie B4 viruses	[154]
Musca domestica	*Musca domestica* antifungal peptide-1 (MAF-1)	Potent antifungal activity against *Candida albicans*	[155]
	MAF-1A analogs viz. Mt6 and D-Mt6	Antibacterial action against *Acinetobacter baumannii*	[156]
Protophormia terraenovae	Phormia A and B	Antibacterial action against Gram +ve bacteria	[157]
Sarconesiopsis magellanica	Sarconesin, Sarconesin II	Antibacterial action against Gram +ve and Gram -ve bacteria; Antiprotozoal activity against *Leishmania panamensis*	[158–160]

effective dosages of ES products pertaining to helminths of human origin, and determine the effectiveness of each product against myriad autoimmune diseases and allergies. Analogues of natural immunomodulatory proteins

Table 5 Indications for leech therapy in humans and animals

Organ Systems	Indications in humans	References	Indications in animals	References
Cardiovascular System	Cardiac hypertrophy and fibrosis, myocardial infarction, stroke	[168, 175]		
Circulatory System	Venous congestion, thrombosis, phlebitis, varicose veins, thromboangiitis obliterans (Buerger's disease)	[173]	Polycythaemia Vera, haematoma	[176, 177]
Digestive System	Gastric ulcers, cholecystitis, hepatitis	[178]		
Endocrine System	Diabetes mellitus and its complications	[179]	Diabetes mellitus	[180]
Excretory System	Urologic trauma, urethral stricture	[181]		
Integumentary System	Cutaneous Leishmaniosis, scabies, eczema, cellulite, atopic dermatitis	[182–184]	Eczema	[185]
Musculoskeletal System	Osteoarthritis, sialadenosis, paradontitis, oroantral fistula	[168, 186]	Laminitis, Arthritis	[187]
Nervous system	Myasthenia gravis, meningococcal purpura fulminans	[168, 188]	Diseases of spine	[185]
Reproductive System	Fibromastopathy, endometriosis, mastitis, prostate cancer	[182]	Endometritis, Penile haematoma, Testicular reperfusion	[189–191]
Respiratory System	Acute rhinopharyngitis, Asthma	[192]	Eosinophilic bronchopneumopathy	[193]

in the form of recombinant proteins can be produced for use in human clinical trials, with the ultimate purpose of developing anthelminthic drugs and vaccines. Helminth therapy should be also tested in farm and companion animals.

MDT and hirudotherapy are following in the footsteps of helminth therapy. The major challenges of MDT are the identification of appropriate fly species other than *L. sericata* and the implementation of this therapy in human trials. While MDT has undoubtedly been valuable in treating chronic ulcers and necrotic wounds in humans and animals, the stigma and psychological impacts of using live maggots may present obstacles to its broader acceptance. Alternatively, with technological advancements and expanding scientific horizons, the future may hold the use of recombinant maggot-derived bioactive molecules being used in wound dressings. The same challenges can also be applied to leeches. There is a general lack of public awareness regarding the benefits of MDT and leech therapies, which need to be popularized among patients, especially in developing countries.

Considering the rising resistance to antimicrobial, anthelmintic, acaricidal, and antiprotozoal drugs, which appears to be irreversible, alternative traditional therapies are urgently needed to counteract myriad ailments. We anticipate that research into parasite-based therapies, still in its infancy, holds great promise.

Authors' contributions

E.S. and S.V. conceptualized the idea of the review; E.S. searched the literature and wrote the manuscript draft; D.S. organized the tables; S.V., D.S. and D.P. edited and revised the manuscript. All authors read and approved the final manuscript.

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