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Necatoriasis: treatment and developmental therapeutics

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Two hookworm parasites, *Necator americanus* and *Ancylostoma duodenale*, infect approximately one billion people worldwide. These hookworms are one of the leading causes of iron-deficiency anaemia especially in children, resulting directly from intestinal capillary blood loss following the feeding activities of fourth-stage (L_4) larva and adult worms. If ignored, human hookworm infections can retard growth and the intellectual development of children. Another clinical manifestation often associated with hookworm infections is cutaneous larva migrans (CLM). It is a well recognised, usually self-limiting condition caused by the infectious larvae of nematodes, especially *Ancylostoma* spp. CLM is characterised by skin eruption and represents a clinical description rather than a definitive diagnosis. Of the hookworm parasites, the dog and cat worm *A. braziliense* and *A. caninum* are the most common nematodes causing CLM, although many other species have also been implicated. The major subject of this review article will be discussion of the evolution of therapies and treatment of human necatoriasis and the development of experimental infections with *N. americanus*. Difference in the clinical efficacy of mebendazole and albendazole will be discussed along with drug resistance of *N. americanus*.

Keywords: *albendazole, Ancylostoma duodenale, cutaneous larva migrans, drug resistance, iron-deficiency anaemia, ivermectin, mebendazole, Necator americanus, necatoriasis*

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1. Introduction

N. americanus and *A. duodenale* are the causative agents of necatoriasis and ancylostomiasis, two hookworm infections common in man. Together with ascariasis, these hookworm parasites remain the most common intestinal nematodes in the world with significant economic, social and medical impact [1-3].

N. americanus, a hookworm parasite of the family Ancylostomatidae, subfamily Necatorinae, is distinguished by the presence of two chitinous cutting plates in the buccal cavity and fused male copulatory spicules. Adult *N. americanus* parasites attached to the villi of the small intestines will suck blood. The infective larvae of *N. americanus*, by being obligatory penetrators, secrete all mechanistic classes of proteolytic enzymes having two overall pH optima of 6.5 and 8.5 [4]. Hookworm larval secretions were shown to degrade a host of human skin macromolecules including collagen types I, III, IV and V, fibronectin, laminin and elastin [4,5]. With the exception of collagen Type V, all other skin macromolecules tested were

hydrolysed by the activity of the *N. americanus* aspartyl proteinase. The latter, in turn, is inhibited by pepstatin A [4].

Utilising a number of different but complimentary approaches, Brown and Pritchard [6] have demonstrated the immunogenicity of the *N. americanus* acetylcholinesterase in infected individuals. Acetylcholinesterase is secreted by wide range of parasitic nematodes including *N. americanus* [7,8]. It plays a potentially parasite-protective role [9] due to the presence of a conserve amino acid sequence in the active site regions of the cholinesterases [10] which allows for human acetylcholine to be a suitable substrate for the parasitic acetylcholinesterase.

Using adult *N. americanus* originating from Togo (Africa) and Sarawak (Malaysia), Romstad *et al.* [11] have found differences in the length of the nucleotide sequences of the second internal transcribed spacer of parasitic rDNA, suggesting that there is either population variation in the sequence of *N. americanus*, or the more likely conclusion is that *N. americanus* from the two countries may have represented genetically distinct but morphologically similar (i.e., cryptic) species.

In general, if lesser parasitic burden is present, the resulting necatoriasis is asymptomatic. The same may also be the case during larval pulmonary migration. However, symptomatic pulmonary disease may occur as consequence of conditions such as Löffler's syndrome, the effects of larval tissue migration, airway reactivity or bronchospasm, infectious bacterial complications from parasitic migration and associated aspiration and rarely, from chronic eosinophilic pneumonia, transdiaphragmatic penetration, or symptoms of upper airway obstruction. It is manifested by fever, cough, chest pain, haemoptysis, dyspnea and wheezing [2]. The most serious clinical manifestation associated with human necatoriasis is iron-deficiency anaemia [12]. To this end, the intestinal blood loss caused by hookworms is proportional to the number of adult parasites in the gut. Even though, as compared to ancylostomiasis, anaemia resulting from necatoriasis is generally considered to be less severe, there has been no preponderance of evidence to suggest that endemic *A. duodenale* infection has had greater impact than *N. americanus* infection on the iron status of the affected populations [13].

Between the asymptomatic state and the presence of iron-deficient anaemia, patients with necatoriasis may display a host of symptoms that include

- cutaneous manifestations of pruritis and dermatitis (commonly referred to as 'ground itch') which develop after penetration of infective larvae through the skin
- pulmonary manifestations such as cough, wheezing, bronchitis, or pneumonitis which occur during larval migration through the pulmonary tree to the gastrointestinal tract
- gastrointestinal manifestations such as abdominal discomfort often with postprandial accentuation, nausea, vomiting, diarrhoea (usually with melena) and cramps, anorexia and the loss of weight

Hypochromic microcytic anaemia may occur in the advanced stage of the disease. Srinivasan *et al.* [14] have observed a significant negative association between *N. americanus* ova load and the haemoglobin levels; the decrease in haemoglobin for a doubling of the ova was estimated by regression analysis to be 0.18, 0.29 and 0.16 g/dl in adult males, females and children, respectively.

Usually, the term 'hookworm disease' is applied when symptoms are present, whereas 'hookworm infection' reflects more or less an asymptomatic state.

Human necatoriasis is prevalent in the Americas as well as in the tropical regions of Africa, southern Asia and Polynesia [15]. Infections by intestinal nematodes would most likely occur in regions with warmer or tropical climate, in rural environments with sub-optimal sanitation, reduced personal hygiene and inadequate education [2]. The hookworm is generally transmitted by larval penetration of the skin.

While the prevalence of necatoriasis will increase with age in children (usually reaching a plateau in late adolescence), the intensity of infection may continue to increase throughout adulthood [12]. In this regard, two factors can determine the intensity of necatoriasis, namely, the degree of exposure to infective larvae and the survival of the parasites within the host. An effective immune response would decrease the survival of the hookworm thereby lowering the intensity of infection and reducing pathology.

1.1 Immunity to *N. americanus*

Maxwell *et al.* [16] examined the clinical and immunological responses of normal human volunteers to

low-dose *N. americanus* (50 infective larvae) infection. All volunteers have developed significant eosinophilia that peaked between days 38 and 64 and ranged from 1350 - 3828 eosinophils/mm^3. Small increases in total and parasite-specific IgE and IgG were observed in some patients, while one volunteer showed a significant lymphocyte blastogenic response. With the exception of mucosal erythaema, bronchoalveolar lavage results were ordinary. These and other data have indicated that small inoculum of *N. americanus* larvae is capable of producing a pronounced clinical effect such as transient gastrointestinal morbidity [16-18]. Furthermore, with the exception of consistent and dramatic rise of blood eosinophil levels, there were no prominent T-cell- and B-cell-dependent immune responses. Direct examination of T-lymphocyte-mediated responses (as assessed by lymphocyte blastogenic technique) did not yield clearly defined results and the total and parasite-specific IgE responses were minimal [16,19-22]. However, the observed low levels of total IgE seen by Maxwell *et al.* [16] contrasted sharply with those seen in patients from endemic areas where much higher levels have been recorded [22-25]. The observed difference in the IgE levels may be the result of factors such as heavy repeated infections of individuals living in endemic regions and the inability to exclude concurrent or prior infections of those subjects with other diverse helminthic parasites [16].

Pritchard *et al.* [26] reported that the natural infection of a community with *N. americanus* has induced a vigorous humoral response to both larval and adult parasite antigens in all five human antibody isotypes. However, at the population level the isotypes responded differently, following chemotherapy and during re-infection, to changes in antigen stimulation. It has been postulated that the observed difference in isotype response probably reflected the fact that the parasite, during the course of its life cycle, presented different amounts of antigens at different anatomical locations. Furthermore, it has been suggested that IgG and IgM responses against adult excretory-secretory (ES) products most accurately reflected the efficacy of chemotherapy and the load of resident adult infection, whereas the IgG responses against larval somatic antigens reflected continuous exposure to infection [26].

Results from studies on an endemically infected population in Papua New Guinea have shown a correlation between antibodies to stage-specific antigens of *N. americanus* and parasite burdens. Thus, using an age-structured analysis, Quinnell *et al.* [27] have demonstrated that the correlation coefficient between levels of IgE against the adult hookworm excretory-secretory antigen and parasite burdens declined significantly with the host age, from positive in younger hosts to significantly negative in older hosts. Similar response patterns were observed for antilarval IgG (both pretreatment and re-infection) and anti-ES IgM and anti-ES IgE pretreatment. The observed patterns were consistent with a role for these isotypes in a protective immune response, although parasite-induced immunosuppression may provide an alternative explanation [27].

In further studies, IgE-rich plasma from Papua New Guinea patients infected with *N. americanus* has been used to probe an adult *N. americanus* cDNA library for the presence of hookworm allergens [28]. One of the allergens, identified as calreticulin, was subsequently expressed in *Escherichia coli*. Even though in small degree, some serological cross-reactivity has been observed between recombinant calreticulin and its host.

The effect of the humoral immune response on the weight and fecundity of *N. americanus* has been examined in an endemically infected population [29]. The results have shown evidence for an effective human immune response to *N. americanus* as demonstrated by the presence of a highly significant negative correlation between total IgE levels and the parasite weight and fecundity, after accounting for any effects of host age and hookworm burden. This correlation was present both at initial treatment and after two years' re-infection. There was a similar negative correlation between the number of eosinophils and hookworm weight and fecundity at initial treatment. It has been postulated that the total IgE levels reflected the level of Th2 cell activation; a Th2-depended immune response acted to reduce the weight and fecundity of *N. americanus* [29].

Palmer *et al.* [30] presented evidence to show that isotype-specific responses, such as IgG4, correlated positively and significantly with both the egg counts and worm burden and may be used as useful markers for the intensity of hookworm infections. In another study, Pritchard and Welsh [31] have found that the specificity of the human IgE response to *N. americanus* may be beneficial in a diagnostic sense, in that antibodies of this isotype have shown minimal cross-reactivity against antigens from the parasite tested.

Results by Olatunde and Onyemelukwe [32] have supported the hypothesis of the existence of immunosuppression of cell-mediated immunity [33,34] in patients with hookworm infection; both the late rosette forming T-lymphocytes and the leukocyte migration inhibition factor levels were low in hookworm-infected patients, especially in those with heavy worm load and marked anaemia.

1.2 Differentiation between *N. americanus, A. duodenale* and other worms

Although traditionally considered to be identical for treatment purposes, there are considerable life history differences between *A. duodenale* and *N. americanus* that should be taken into consideration prior to rational design of chemotherapeutic and immunoprophylactic control strategies. Applying a PCP-based technique, Hawdon [35] was able to amplify a fragment of the 3′ untranslated region of the cAMP-dependent protein kinase catalytic subunit gene from both *A. duodenale* and *N. americanus* genomic DNA by using primers derived from the corresponding *A. caninum* cDNA. The polymerase chain reaction-linked restricted fragment length polymorphism (PCR-RFLP) technique can distinguish between pure and mixtures of hookworm DNA and can amplify DNA from a single egg [35].

Using the PCR-RFLP analysis, Romstad *et al.* [36] have demonstrated that *Oesophagostomum bifurcum* and *N. americanus* adult worms could be readily differentiated from one another based on the size difference of PCR products and by their specific internal transcribed spacer (ITS) patterns. It should be noted that human oesophagostomiasis caused by *O. bifurcum* (a nodular worm) is of major health concern in northern Togo and Ghana, where *N. americanus* also exists at high prevalence.

1.3 Cross-reactivity between *N. americanus, Schistosoma mansoni* and *Ascaris lumbricoides*

Polyparasitism is commonly observed in endemic communities and reactivity of sera from hookworm-infected patients against schistosomular and ascaris antigens has also been reported. The protective cross-immunity between *N. americanus* and *Schistosoma mansoni* has been investigated in NIH and BALB/c mice and its implication with respect to sero-diagnosis has been discussed by Timothy *et al.* [37]. While protective resistance to homologous challenge with both parasites has been confirmed, functional immunity to heterologous challenge was not demonstrated. Furthermore, sera from mice that had received homologous challenges with *N. americanus* and from hookworm-infected animals, which had previously been exposed to radiation-attenuated *S. mansoni*, exhibited an enhanced IgGA/IgGM response to infective stage *N. americanus* somatic antigens.

After studying a population from Papua New Guinea infected predominantly with *N. americanus*, Pritchard *et al.* [38] have presented sero-epidemiological evidence demonstrating the presence of a high degree of cross-reactivity between IgG, IgA and IgM antibody responses to *N. americanus* ES and *Ascaris lumbricoides* PCF antigens. The results suggested that the cross-reactivity was due to epitopes carried on a range of *Ascaris* antigens and that a number of *Necator*-specific antigens do exist. The observed cross-reactivity accounted for a peak in the antibody levels against *N. americanus* in 10 - 13 years old children (driven by infection with *A. lumbricoides*) as well as in the maintenance of apparent antibody levels against *A. lumbricoides* in older age groups (driven by infection with *N. americanus* in the absence of overt infection with *A. lumbricoides*).

2. Evolution of therapies and treatment of necatoriasis

The potent antiparasitic activity of two benzimidazole drugs, mebendazole (5-benzoyl-2-benzimid azolecarbamic acid methyl ester) and albendazole (methyl 5-propylthio-2-benzimidazolecarbamate) has been used very effectively against human hookworm infections [39-48]. The relatively low cost of mebendazole [49] has been an important factor to consider, especially in the treatment of hookworm infestations in impoverished communities.

Initial studies indicated that albendazole may have elicited its antihelminthic activity by selectively blocking the glucose uptake by adult worms lodged in the intestine and their tissue-dwelling larvae [50]. Inhibition of the glucose uptake is thought to result in the endogenous depletion of glycogen stored within the parasite thereby causing a decrease in the formation of adenosine triphosphate, which is essential for the reproduction and survival of the parasite. Recent developments, however, have demonstrated that benzimidazole anthelminthics may exert their antiparasitic activity *via* binding to the nematode β-tubulin. By binding to free β-tubulin,

benzimidazoles inhibit the polymerisation of tubulin and the microtubule-dependent uptake of glucose [51,52]. This mechanism of action is believed to account for the association between tubulin alleles and drug resistance.

Albendazole appeared to be more effective than mebendazole in hookworm infections [53], due mainly to its high efficacy in field trials [53-55] and compliance under local conditions. However, a single 500 mg dose of mebendazole has been recommended as the treatment of choice for human hookworm infection [49,56,57]. Another regimen consisting of twice daily oral administration of 100 mg of mebendazole for three days has shown cure rates in the 70.3 - 93% range [58].

Nontasut *et al.* [59] investigated the efficacy of mebendazole administered as single or multiple-dose regimens in doses lower (25, 50 and 75 mg) than 100 mg. In the reported study, one group of patients also received conventional dose of mebendazole (100 mg twice daily for 3 days) and another group was treated with a standard dose of albendazole (400 mg as a single dose). The results have demonstrated that all three lower doses (75, 50 and 25 mg) of mebendazole were highly effective against *A. lumbricoides*, but only moderately effective against *N. americanus* with cure rates of 64%, 48.6% and 35.3%, respectively.

In a trial conducted in 66 children (4 - 14 years of age) with mild-to-moderate hookworm infection (*N. americanus* in 99% of cases), two mebendazole regimens: 600 mg (conventional treatment of 100 mg b.i.d. for 3 days) and 300 mg (as a single dose), were compared for efficacy [60]. The cure rates for the 600 mg and 300 mg regimens were 16.1% and 91.4%, respectively; the corresponding values for egg reduction rates were 90.9% and 99.5%, respectively. Chavarria *et al.* [61] reported a cure rate of 44.4% after administration of a single 300 mg dose of mebendazole. Previous data by Cabrera [62] described a cure rate of 88.2% after mebendazole was given as a 600 mg single dose [63]. While the latter result may imply that a higher dose of the drug even when administered as a single dose is more effective, Holzer and Frey [64] have reported that a single 1.0 g oral dose of mebendazole cured only 21% of patients infected with *N. americanus*.

A combination of 150 mg mebendazole and 30 mg pyrantel pamoate given on three consecutive, or near-consecutive, days to children infected with *N. americanus* has proven effective in 95% of the cases [65].

Another broad spectrum anthelminthic, albendazole has also been reported to be effective in the treatment of necatoriasis when given as a single dose of 400 mg preferably for three days [45-48,54,66-71]. Detectable plasma levels of the sulphoxide metabolite of albendazole (average half-life of 8 - 9 hrs) were found within 30 min of dosing [50,72]. The observed side effects of albendazole (abdominal pain, nausea, vomiting, alopecia, increased serum aminotransferase and neutropenia) usually would not require cessation of therapy [52]. Its teratogenicity has not been extensively studied; however, as with all benzimidazole drugs; the drug should be avoided whenever possible in women of childbearing age [52].

Similarly to mebendazole [73,74], albendazole has also exhibited ovicidal effect against hookworms; at a 400 mg single oral dose it suppressed the hatching of *N. americanus* eggs [67]. The drug has been well-tolerated and minimal clinical side effects have been reported. The expulsion rates showed that most worms (about 90%) were expelled on the third day after treatment [59].

Twelve weeks after treatment of pre-school children infected with *N. americanus* with a single oral dose (400 mg) of albendazole (administered twice at an interval of 14 weeks), the re-infestation rate was only 3%, as compared to 16% for *Ascaris lumbricoides*, 33% for *Truchuris trichiura* and 24% for *Giardia lamblia* [64]. Pamba *et al.* [75] have described 100% cure rate against *N. americanus* infection in children less than two years old after receiving a single dose of 200 mg (10 ml) of albendazole suspension.

Sacko *et al.* [76] conducted a randomised, placebo-controlled trial in the southern region of Mali to compare the efficacies of mebendazole (500 mg single dose), albendazole (400 mg single dose) and pyrantel pamoate (12.5 mg/kg, single dose). The patients were re-examined 10 days after treatment and after controlling for the drift in faecal egg counts in the placebo-treated subset, age, gender, fasting and intensity of infection. The results have demonstrated that albendazole was clearly the most effective drug showing consistently efficacy in the 92.1 - 99.7% cure range, depending on the method of evaluation and the particular subset of the treatment group. Neither mebendazole nor pyrantel pamoate have been effective, showing efficacy in the 60.9 - 89.8% and 4.8 - 89.7%, respectively. Fasting made no difference to

drug efficacy. Overall, a single dose of 400 mg of albendazole was the treatment of choice for hookworm infection in this region of Mali.

When used against hookworm infections, a standard regimen of pyrantel pamoate is applied usually as a single oral dose of 11 mg/kg, to a maximum dose of 1.0 g [35].

Ivermectin, the 22,23-dihydro analogue of the macrocyclic lactone avermectin B_1, has displayed antiparasitic activity *in vitro* [77] and against an array of nematode and arthropod parasites in veterinary medicine [78-80]. When administered as a suspension into to the stomach of hamsters by oral intubation, ivermectin at 30 mg/kg completely cleared pre-adult *N. americanus*; a regimen consisting of 10 mg/kg (followed by a repeated dose on day later) achieved the same result [81]. However, hamsters carrying adult *N. americanus* worms were completely cured of infection by doses of 15 mg/kg (single dose) and 7.5 mg/kg (repeated on the next day).

Xia *et al.* [82] studied the clinical efficacy of ivermectin in the treatment of several human nematode infections. As compared to ascariasis and trichuriasis, the therapeutic efficacy of ivermectin against hookworm infections (*N. americanus* and *A. duodenale*) was rather disappointing. Thus, when tested at single oral doses of 0.1 and 0.2 mg/kg, the cure rates against ascariasis, hookworm and trichuris infections were 100%, 3.8% and 50% and 95.5%, 11.8% and 76.5%, respectively. By comparison, pyrantel pamoate at a single oral dose of 10 mg/kg showed cure rates of 95.5%, 29.6% and 31.6%, respectively. The observed side effects were mild and transient in all groups [82]. Naquira *et al.* [83] also reported little therapeutic efficacy of ivermectin in patients with hookworm infections (*A. duodenale, N. americanus*) following medication with 50, 100, 150, or 200 µg/kg as a single dose, or a two-day course with either 100 or 200 µg/kg of the drug. Similarly, Whitworth *et al.* [84] reported no apparent effect of ivermectin on *N. americanus* infection in man when tested at 150 mg/kg in a double-blind placebo-controlled trial conducted in Sierra Leone.

The effects of a new anthelminthic drug, tribendimidin {*N,N*-[bis-4'-(1-dimethylaminoethylidene amino)phenyl]-1,4-phenylenedimethylidyne amine} on the cuticle of *N. americanus* was evaluated in golden hamsters [85]. One hour after medication with a single dose of 150 mg/kg, some worms showed cuticular swelling, fusion of transverse striations and attachment of host leukocytes on the parasite's damaged cuticular surface. At four hours post-treatment, the cuticle revealed moderate swelling or even erosion; the ventral cutting plates appeared to be swollen. After 8 - 24 hrs, severe cuticular swelling, erosion and peeling in female worm tails and male copulatory bursa, were observed. After 4 - 8 hrs post-treatment, there has been no increase in lesions in the small intestinal mucosa of the hamsters. The histological and histochemical effects of tribendimidin on *N. americanus* have also been studied by the same investigators [86].

The *in vitro* response of adult (males and females) and free-living stages of *N. americanus* to a wide spectrum of anthelminthics was assessed by Kumar [87]. The observed activity varied from 0.0002 and 0.0007 mg/l for pyrantel pamoate and tricofenol piperazine, respectively, to about 8.47 and 7.6 mg/l for morantel tartrate and amoscanate, respectively. An overall conclusion was drawn that neither sex nor life cycle stage alone may serve as effective criteria for screening of anthelminthic agents. Furthermore, female parasites should be taken into account for the assessment of ED_{50} values since they had required relatively the highest ED_{50} levels in nearly all of the compounds studied.

2.1 Differential efficacies of mebendazole and albendazole

Nontasut *et al.* [45] have conducted comparative studies on the clinical efficacy of orally administered mebendazole and albendazole. The results have shown that a single 400 mg dose of albendazole was superior to a single 600 mg dose of mebendazole (cure rates of 64% and 11%, respectively) in the treatment of *N. americanus* infections in Thailand. Increasing the single dose of albendazole to 600 mg and 800 mg did not significantly alter the cure rate of the drug [47,48,64]. Increasing the dose of mebendazole to 1.0 g also seemed not to improve the cure rate against hookworm infections [64]. These results are in contrast to those of Cauwenberg [88] that showed that increasing the single dose of mebendazole from 200 mg to 500 mg and to 600 mg yielded cure rates of 8%, 54% and 78%, respectively. One possible explanation for the reported discrepancies may lie in the different way cure rates have been measured.

Vandepitte [89] has presented clinical evidence demonstrating that when a lower total dose of 600 mg

mebendazole is administered as a three-day course for the treatment of hookworm infections, the cure rate exceeded 90%. This may indicate that the duration of exposure of the parasite to mebendazole rather than the concentration of the drug in the bowel is important for efficacy. However, as shown by Holzer and Frey [64], this was not the case for albendazole.

The reason for the difference in the clinical efficacy of mebendazole and albendazole is not very clear [64]. It may be due to the different pharmacokinetic properties of the two drugs. For example, while albendazole is well absorbed from the gastrointestinal tract and has systemic effect [90], mebendazole is poorly absorbed and has mainly intraluminal activity [91].

2.2 Drug resistance of *N. americanus*

Preliminary results from a placebo-controlled randomised trial conducted in Mali have shown that a single dose of 500 mg mebendazole was ineffective in the treatment of 103 patients infected with *N. americanus* [92]. Thus, there has been less than significant reduction of the parasite burden as assessed by faecal egg counts (18.5% eggs per gram of faeces). This failure of mebendazole to treat necatoriasis in the southern region of Mali is indicative that, among the other possibilities, the emergence of resistance to mebendazole by *N. americanus* may have contributed to drug failure [92]. By comparison, treatment with pyrantel pamoate (10 mg/kg) led to a marked reduction (75%) of faecal egg counts. Among other possibilities, the results may suggest the development of resistance to benzimidazole agents by *N. americanus*. It should be noted that benzimidazole resistance is widespread among nematode parasites in domestic animals. There have also been pronounced regional differences with extensive drug resistance being reported in South Africa, New Zealand and Australia [93,94]. Among the possible reasons for the development of resistance towards benzimidazoles, too frequent treatment, the use of inappropriate doses and the failure to provide alternative treatment strategies with other drugs are believed to be responsible for the resistance to specific anthelminthics [95,96]. It has been shown that resistance to benzimidazoles due to a loss of the drug's high affinity for binding to tubulin [52] had developed in intensively drug-treated livestock.

While little is known about selection pressure for appearance of drug resistance among human nematodes, studies on ovide parasites have shown that only a few rounds of treatment, at a time when most parasites are in their hosts, selected strongly for resistance [97]. Even though drug resistance may be slow to appear [98], it has long been anticipated because of the widespread misuse of anthelminthics in the therapy of human infections [97,99]. It is also possible that *N. americanus* in different localities may differ in its sensitivity to anthelminthics (e.g., mebendazole) due to genetic drift among the parasites relative to other geographically distant hookworm populations [92].

Among the other hypotheses for drug resistance that were proposed, different drug formulations, such as size particles, may have been responsible for the observed lack of activity [100]. In addition, Weshe *et al.* (101) have attributed the contrasting efficacy of two formulations of mebendazole to the efficiency of tablet breakdown in aqueous medium.

3. Hookworm infections and iron-deficiency anaemia

Hookworm infections in man, especially in children, are one of the leading causes of iron-deficiency anaemia [1,13,102-104] resulting directly from intestinal capillary blood loss following the feeding activities of fourth-stage larva and adult worms [105]. During the attachment of adult hookworms to the gastrointestinal mucosa, intestinal capillaries of the lamina propria are lacerated and the extravasated blood is either ingested by the fastened worm or will leak at the site of the parasite attachment [106]. The use of radioactive tracers [107] has allowed investigators to estimate that 30 µl of blood daily is lost to an individual *N. americanus* and 260 µl to *A. duodenale* [108,109]. Tissue damage and collagen exposure would normally trigger haemostasis. However, hookworms have evolved strategies to prevent haemostasis [110,111], such as the ability of *N. americanus* to inhibit Stypven clotting time and the coagulation Factor Xa (a pivotal component of the clotting cascade) [112-116]. Another important factor contributing to parasite survival is the ability of adult hookworms to interfere with platelet aggregation and activation, as well as to degrade fibrinogen [12,114,117-129]. These complementary antihaemostatic strategies coupled with the accumulation of worms throughout the lifetime of the host [130] have enabled the parasites to establish a chronic infection

commonly observed in human populations. Finally, the cumulative blood loss will usually lead to iron-deficiency anaemia in the infected individual [130].

Anaemia resulting from iron deficiency is a slowly developing condition that is known to proceed through progressive states of pre-latent, latent and manifest iron deficiency [131,132]. The pre-latent iron deficiency involves the loss of sequestered iron reserves (storage iron depletion) without a decrease in the iron supply to the developing red cells. The second latent stage (also referred to as 'iron-deficient erythropoiesis') is a corollary of diminished erythroid iron supply and occurs in the absence of a significant effect on circulating haemoglobin levels. The continued blood loss and lack of iron replacement then lead to the development of the third stage, manifested as iron-deficiency anaemia. All three stages can be monitored respectively by measuring the levels of serum ferritin, free erythrocyte protoporphyrin and haemoglobin [131,132].

In a number of studies, the relationship between hookworm infection and anaemia has been established by using hookworm egg production as an indirect method to measure the worm burden. Such studies have shown the degree of anaemia to be related to hookworm egg production, at least when egg counts exceeded 5000 eggs/g faeces [108].

Pritchard *et al.* [133] assessed the relationship between the iron status and the intensity of infection by monitoring the effect of *N. americanus* burden on the haemoglobin, haematocrit and the serum ferritin levels. The results have demonstrated the presence of a significant negative correlation between the plasma ferritin level and the hookworm burden, which was most pronounced in male patients. In contrast, there has been no correlation between plasma ferritin and hookworm egg count and no consistent correlation between the haemoglobin level or haematocrit and either measure of hookworm intensity. The overall results have indicated that the role of hookworm (*N. americanus*) in the aetiology of anaemia may be difficult to assess without the accurate measurement of the hookworm burden.

Stoltzfus *et al.* [134] have measured the intensity of hookworm infection using faecal egg counts, whereas gastrointestinal blood loss was measured by determining the concentration of haem in the faeces utilising the Hemoquant method [135]. The latter is used to determine the amounts of porphyrins in the faeces before and after digestion that degrades haem to its porphyrin constituents. Next, the concentration of porphyrin after digestion is used to calculate the total faecal haem concentration. The ratio of the porphyrin concentration before and after digestion will indicate where the blood loss occurred in the intestinal tract. A high proportion of porphyrin in the faeces before the digestion process will indicate blood loss higher in the intestinal tract [12].

It is estimated that a concentration of 10 mg/g faecal haem represents a daily loss of > 2.0 mg of iron, which is more than double the median iron requirement of a healthy school-age child [12]. In a disturbing trend, in children whose faecal haem exceeded 10 mg/g faeces, 100% had serum ferritin levels < 18 μg/dl, 93% had haemoglobin concentrations of < 110 g/l and 29% had haemoglobin concentrations < 70 g/l [134]. In this particular sample of children, the predominant hookworm was *N. americanus*, with about 10% having *A. duodenale*. However, when compared, blood loss from *A. duodenale* has been 2 - 10 times higher than that caused by *N. americanus* [108].

Genta and Woods [136] have described a case of a patient with severe iron-deficiency anaemia in whom adult hookworm females were visualised, recovered and speciated during an upper endoscopic procedure.

By drawing attention to the importance of the regulation of stored iron levels in the process, Crompton and Whitehead [137] proposed a mathematical model to investigate how hookworm infection might disturb human iron metabolism and to explain how the iron metabolism may respond and adapt to a hookworm infection of varying degree.

4. Cutaneous larva migrans

Cutaneous larva migrans is a well recognised, usually self-limiting condition caused by the infectious larvae of nematodes. CLM is characterised by skin eruption and represents a clinical description rather than a definitive diagnosis [138]. Of the hookworm nematodes, the dog or cat worm, usually *A. braziliense* and *A. caninum* are the most common parasites causing CLM, although other species have also been implicated [139]. Even though CLM is diagnosed simply, its causative organism is not identified since biopsy is not thought to be beneficial [140].

Although *A. braziliense* is the hookworm nematode more responsible for CLM, *N. americanus* has been implicated in several cases of CLM [138,141,142] as well. The usual cutaneous manifestation of infection caused by *N. americanus* is pruritic reaction at the site of inoculation known as 'ground itch', followed within hours by an erythematous papular or papulovesicular rash [139]. The cutaneous symptoms will usually subside as the larvae penetrate the skin and migrate through the venous system into the lungs [138].

Topical treatment with thiabendazole suspension has been widely used because of its efficacy and safety [143]. Thiabendazole is usually applied at 25 mg/kg twice daily (to a maximum of 3.0 g per day) for 2 - 5 days in adults and children [3].

Loughrey *et al.* [138] have applied a three-day course with oral albendazole (400 mg daily) to successfully treat CLM caused by the filariform larvae of *N. americanus;* the symptoms were resolved completely within two weeks and the patient suffered no side effects [144,145]. However, recurrence after a three-day course has been reported to occur due to heavy infestation [146]. By comparison, oral thiabendazole although equally effective, has shown more side effects, including nausea, vomiting and dizziness [147]. Ivermectin was also used in the therapy of CLM with cure rate as high as 100% with no toxic side effects [148,149].

5. Expert opinion

The global occurrence of hookworm infections by some estimates [97,150] reaches 900 million people. These infections are responsible for the development of about 1.5 million cases of iron-induced anaemia (hookworm anaemias), representing roughly one-third of all iron-deficiency anaemias to be of this type. In addition, cases of hookworm anaemias result in 30,000 - 60,000 deaths each year. These figures taken together emphasise the public health importance of hookworm infections as a cause of serious morbidity and mortality, especially in millions of children where they may retard growth and intellectual development; a health problem that has been largely ignored by healthcare providers and researchers.

The highest priority in hookworm control is the reduction of mortality and morbidity by a combination of community-based chemotherapy and vast improvement in sanitation to prevent parasite transmission. Since the 1970s, two benzimidazole anthelminthic agents, mebendazole and albendazole have had established roles in the chemotherapy of hookworm infections in general and necatoriasis in particular. Recently, however, there has been evidence for the emergence of resistance to these two drugs. The latter, coupled with the fact that gaining access to therapy is problematic in many regions of the world and because dormant larvae are able to generate new infections months after chemotherapy has ceased, should provide impetus for renewed efforts towards the discovery of new chemotherapeutic agents. The need for new antihookworm therapies has been made even more acute by the fact that humans afflicted with an initial hookworm infection apparently do not acquire the strong protective immunity against future infections.

Lately, there has also been a renewed interest supported by new findings suggesting excellent possibilities for the discovery and development of vaccines against hookworm infections [1,151]. Thus, the development of genetically engineered vaccines would greatly help in the control of these infections, especially in highly endemic areas. In recent studies, recombinant polypeptides belonging to the *Ancylostoma* secreted protein (ASP-1) family have shown promise for reducing hookworm burdens after larval challenge infections in mice [151]. Since any vaccine is antibody-dependent, it is anticipated that a cocktail of different recombinant hookworm antigens may be required in order to effectively prevent heavy hookworm infections and disease. However, efforts to develop antihookworm vaccine have been hindered by the lack of a suitable animal model of hookworm infection resembling the human disease, as well as the need for easily available native hookworm antigens. In addition, useful serological correlates of antihookworm immunity are still poorly defined [151].

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