31 Salzet, M. *et al.* (1997) Biochemical and molecular characterization of ACTH, its precursor in the leech *Theromyzon tessulatum*: morphine increases ACTH levels. *J. Immunol.* 159, 5400–5411
32 Pearce, E.J. *et al.* (1991) Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth *Schistosoma mansoni*. *J. Exp. Med*. 173, 159–166
33 Lefebvre, C. *et al*. (2000) Peptides opioïdes, substances opiacées et réponse immunitaire. *Med. Sci.* 16, 235–242
34 Weinstock, J.V. and Blum, A.M. (1983) Isolated liver granulomas of murine *Schistosoma mansoni* contain components of the angiotensin system. *J. Immunol.* 13, 2529–2532
35 Salzet, M. and Stefano, G.B. (1998) Evidence for an invertebrate neuroendocrine system: neuropeptide processing in leech–host communication. *Trends Comp. Biochem. Physiol.* 5, 85–98
36 Salzet, M. *et al.* (1999) Serine protease inhibitors: an evolutionary conserved immunostrategy. *Immunol. Today* 20, 541–544
37 Leung, M.K. *et al.* (1995) *Schistosoma mansoni*: the presence and potential use of opiate-like substances. *Exp. Parasitol.* 81, 208–215
38 Laurent, V. *et al.* (2000) Morphine-like substance in leech ganglia: evidence and immune modulation. *Eur. J. Biochem.* 267, 2354–2362
39 Stefano, G.B. *et al.* (1996) Opioid and opiate immunoregulatory processes. *Crit. Rev. Immunol.* 16, 109–144
40 Smith, E.M. and Blalock, J.E. (1981) Human lymphocyte production of ACTH and endorphin-like substances: association with leukocyte interferon. *Proc. Natl. Acad. Sci. U. S. A.* 78, 7530–7534
41 Salzet, M. *et al.* (2000) Comparative biology of the endocannabinoid system: possible role in the immune response. *Eur. J. Biochem.* 276, 4917–4927
42 Dissous, C. *et al.* (1986) *Schistosoma mansoni* shares a protective oligosaccharide epitope with freshwater and marine snails. *Nature* 323, 443–445
43 Grzych, J.M. *et al*. (1987) *Schistosoma mansoni* shares with keyhole limpet hemocyanin a protective carbohydrate epitope. *J. Exp. Med*. 165, 865–878
44 Davies, S.S. *et al*. (1998) A divergent member of the transforming growth factor B receptor family from *Schistosoma mansoni* is expressed on the parasite surface membrane. *J. Biol. Chem.* 273, 11234–11240
45 Auriault, C. *et al.* (1985). Regulatory role of a tripeptide (TKP) from the second constant domain of immunoglobulin G - I. Inhibition of rat and human macrophage activities. *Int. J. Immunopharmacol.* 7, 73–79
46 Auriault, C. *et al.* (1981). Interaction between macrophage and *Schistosoma mansoni* schistosomula: role of IgG peptides and aggregates on the modulation of β-glucuronidase release and the cytotoxicity against schistosomula. *Cell. Immunol*. 62, 15–19

Geographical Variation in *Ascaris lumbricoides* Fecundity and its Implications for Helminth Control

A. Hall and C. Holland

The observation by microscopy of nematode eggs in human faeces is used to diagnose a helminthic infection, while the concentration of those eggs is used to estimate the number of worms in the host. Within a community, the prevalence of infection and the mean egg count provide useful information about the extent of a public health problem, and are being used to guide the growing efforts to control disease caused by helminths. Here, Andrew Hall and Celia Holland examine data on the relationship between the worm burdens of Ascaris lumbricoides *and the concentration of eggs in faeces, and discuss the implications of the variation found for using such data to plan helminth control programmes.*

Intestinal nematodes are very common human parasites and just one species, *Ascaris lumbricoides*, is estimated to infect a quarter of the world's population[1]. Because sexually mature female worms produce characteristic eggs, infections can be diagnosed by direct microscopy of faeces. However, this diagnosis does not indicate whether the infected person is diseased, because morbidity is related to the number of worms living in the intestine[2]. Because the number of worms cannot be determined without expelling them from the gut, parasitologists use the concentration of eggs in faeces as an indirect estimate of the worm burden. This involves many assumptions as well as potential sources of inaccuracy. For example, only females produce eggs, so an even sex ratio is assumed. In addition, the concentration of eggs can vary within a faecal sample and from day to day by an amount that is difficult to explain by the dilution of eggs in faeces[3]. Nevertheless, when data are aggregated for a reasonable sample of subjects, the mean concentration of eggs in faeces is generally considered to be representative of the worm burden. This was recognized in 1987 by a WHO Expert Committee, which defined a light infection with *A. lumbricoides* as <5000 eggs per g faeces (epg) and a heavy infection as >50 000 epg[4]. Thresholds have also been proposed for *Trichuris trichiura* and the hookworms, and are now being used to guide programmes to control soil-transmitted helminths[5].

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Current helminth control strategies

The strategy of recent helminth control programmes has been to prevent disease, rather than infection, with the aim of reducing worm burdens below a level associated with morbidity, and then sustaining that low level[6]. The development of safe, effective and inexpensive anthelmintics, such as albendazole and mebendazole, has led to recommendations that periodic mass treatment can be given where the prevalence of intestinal nematodes in schoolchildren is >50%[7]. In some circumstances, however, the prevalence can rebound within six months almost to pretreatment levels[8,9]. This has led to the proposal in two recent WHO publications that mass treatment should be given two or three times a year when >10% of the target group has high-intensity infections[7,10]. For *A. lumbricoides*, the threshold proposed for a high-intensity infection is 50 000 epg[4].

Since this threshold was published in 1987, there have been several investigations that have measured worm burdens of *A. lumbricoides* after anthelmintic

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treatment. These include two large studies in Nigeria[11] and Bangladesh[12], and we have used these data to examine the relationship between worm burdens and faecal egg counts. In addition, we have compared these two studies with several others to examine the relationship between worm burdens and the fecundity of female worms.

Table 1. The characteristics of infections with *Ascaris lumbricoides* in school-age children in studies in Bangladesh and Nigeria[a]

Characteristic	Bangladesh	Nigeria
Sample size	836	670
% With eggs in faeces	83.8	87.0
Mean eggs per g faeces (epg)	2473	13 609
Median epg	1182	7862
Maximum epg	30 510	143 433
% Who passed worms at first treatment	90.2	93.3
Mean number of worms	20.2	12.6
Median number of worms	13	9
Maximum number of worms	187	196
% Infections missed by microscopy	6.4	6.3
% Who passed worms after second treatment, six months later	82.2	67.0
% Who passed worms after third treatment, another six months later	84.6	62.0

[a] Data from Refs 11,12.

Nigeria and Bangladesh

Both studies involved schoolchildren aged 5–16 years. Each subject gave a faecal sample for a quantitative examination using the same modified ether sedimentation technique[13], and the concentration of *A. lumbricoides* eggs was expressed in epg. Children in Nigeria were treated with levamisole and in Bangladesh with pyrantel pamoate, both given at the manufacturers' recommended dosage. These are highly effective anthelmintics, which paralyse *A. lumbricoides* so that they are expelled intact by peristalsis. Both drugs are reported to remove all worms in 80–100% of cases[14], and we know of no evidence of resistance by *A. lumbricoides* to these drugs. All the worms expelled for the 48 h following treatment were recovered, sexed and counted.

Table 1 shows the characteristics of the infections with *A. lumbricoides* in Nigerian and Bangladeshi children. The prevalence was very similar in both countries, whether diagnosed by stool microscopy or by expelling worms, and microscopy missed almost the same percentage of infections in each study. Although the mean worm burden in Nigeria was ≈60% of the mean in Bangladesh (12.6 vs 20.2), the mean egg count was over five times higher (13 609 vs 2473).

To examine the relationship between the concentration of eggs in faeces and worm burden, subjects were grouped according to their worm burdens while ensuring an arbitrary minimum of 20 children per group. Figure 1 shows the mean worm burden plotted against the mean egg count and reveals striking differences between countries: for the same worm burdens in both countries, the egg counts in Nigeria were between six and 13 times greater than in Bangladesh.

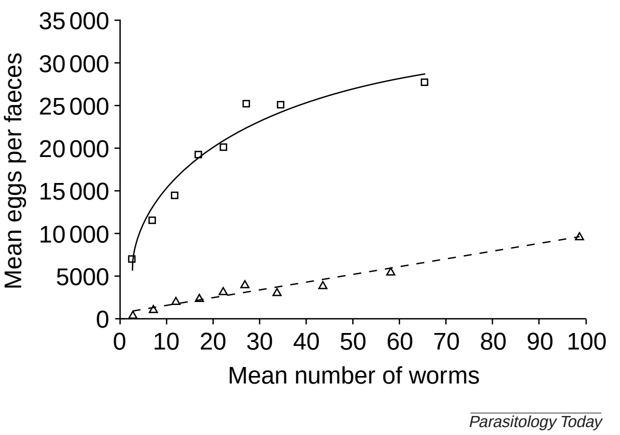

Fig. 1. The relationship between the average worm burden with *Ascaris lumbricoides* in school-age children in Bangladesh (triangle) and Nigeria (square) and the average concentration of the eggs in faeces. The data on worm burdens have been grouped as follows: 1–4, 5–9, 10–14, 15–19, 20–24, 25–29 worms, then in Nigeria 30–39 and ⩾40 worms or, in Bangladesh, 30–39, 40–49, 50–69 and ⩾70 worms. The lines represent the best empirical fits and are linear for Bangladesh ($y = 88.036x + 762.02$) and logarithmic for Nigeria [$y = 7016\mathrm{Ln}(x) - 708.77$].

To examine what this means in terms of light, moderate and heavy infections, the data are presented in Fig. 2 for each country in terms of both the WHO categories of egg counts[4] and, in the absence of similar categories for worm burdens, for three arbitrary categories. Figure 2a reveals that no children would have been classified as heavily infected in Bangladesh based on egg counts and only 15% were moderately infected; these percentages in Nigeria were 4% and 54%, respectively. Figure 2b shows that 13% of children in Bangladesh were classified as heavily infected (⩾40 worms) and 24% were moderately infected (20–39 worms), whereas the same figures for Nigerian children were 3% and 17%, respectively.

If the reason for using an egg count threshold is to define the need for treatment more than once a year because of rapid reinfection, then perhaps it is better to consider using the prevalence of reinfection as an indicator. Table 1 shows that the prevalence in both countries had rebounded within six months to above the 50% threshold, which would warrant mass treatment, and had done so again six months after a second round of treatment.

It seems that, in both Nigeria and Bangladesh, the threshold of 50 000 epg does not reflect the need for treatment every six months, and that the considerably lower fecundity of worms in Bangladesh indicates that any threshold, whatever it is, might not be universally applicable.

Worm fecundity and worm burdens

Because it is difficult to draw conclusions from only two studies, additional data were sought. These were provided by studies of *A. lumbricoides* expelled after treatment in Burma[15], Iran[16], Mexico[17] and northern Bangladesh[18] in which equations for the relationship between worm burden and fecundity were given. In addition, unpublished data were obtained from a study in Madagascar[19]. Table 2 summarizes the seven studies in six countries for which data were examined.

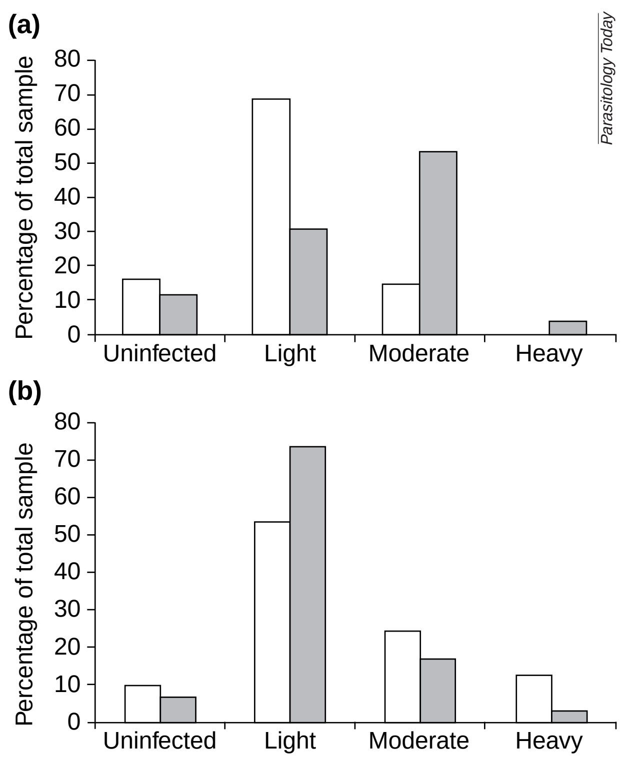

Fig. 2. The classification of infections with *Ascaris lumbricoides* in school-age children in Bangladesh (open bar) and Nigeria (closed bar) according to: eggs per g faeces (epg) as proposed by the WHO as light (1–4999 epg), moderate (5000–49 999 epg) and heavy (⩾50 000 epg)[3] (a); arbitrary categories of worm burden in which light is defined as 1–19 worms, moderate as 20–39 and heavy as ⩾40 worms per person (b).

Figure 3a shows the relationship between fecundity and worm burden for *A. lumbricoides* and reveals several points. First, in all studies, the biggest drop in fecundity occurred in burdens of between one and ten worms. This suggests that the effects on fecundity are not to do with competition for resources or a 'crowding effect' (because they occur at low worm burdens), but are the result of some sort of inhibitory process.

Second, the two studies in Bangladesh, undertaken by different groups several years apart (one in a rural area in the north[18] and one in an urban slum in the capital[12]), found female worms with a similar fecundity. This provides confirmation of the data presented in Fig. 1.

Third, for any given worm burden, there were relatively large differences in worm fecundity. For example, Fig. 3a shows that, for an average of 20 worms per host, the concentration was 200 epg per female worm in Bangladesh compared with 1700–2600 epg per female worm in Burma, Iran, Madagascar and Nigeria, and ≈4400 epg per female worm in Mexico. Figure 3b indicates how these egg counts might translate into worm burdens by simply multiplying the egg output per female worm by the total number of female worms and then dividing by two (assuming an even sex ratio) to account for the male worms. It indicates that, in Mexico, the WHO threshold of 50 000 epg would be reached at a burden of around 25 worms; in Burma, Madagascar and Nigeria at around 100 worms; in Iran at about 260 worms; and not within a biologically possible worm burden at either site in Bangladesh.

The implications for helminth control programmes

The data presented here suggest that the fecundity of female *A. lumbricoides* might vary around the world. It is

Table 2. The characteristics of studies that have examined the relationship between the fecundity of female *Ascaris lumbricoides* and the total worm burden expelled after treatment[a]

Study characteristics	**Bangladesh 1**	**Bangladesh 2**	**Burma**	**Iran**	**Madagascar**	**Mexico**	**Nigeria**
Method used for egg counts	Formalin ether sedimentation	Formalin ether sedimentation	Formalin ether sedimentation and Kato–Katz	Stoll flotation	Formalin detergent sedimentation	Kato–Katz	Formalin ether sedimentation
Anthelmintic used	Pyrantel pamoate	Pyrantel pamoate	Levamisole	Pyrantel pamoate	Mebendazole	Mebendazole	Levamisole
Data analysed for individuals or groups	Groups	Groups	Groups	Groups	Groups	Individuals	Groups
Sample size of individuals	59	1365	239	216	233	34	563
Sample size of groups	8	13	9	9	8	NA	7
Age range (years)	0.5–15	6–15	All ages	All ages	0–11	2–10	6–15
Function fitted to line	Power	Power	Power	Power	Power	Power	Power
Power function[b], *a*	635	291	16 560	9921	9374	9400	9802
Power function[b], *b*	¯0.35	¯0.2737	¯0.61	¯20.584	¯20.485	¯20.250	¯20.4994
Correlation coefficient, *r*	NG	0.891	0.981	NG	0.855	0.484	0.992
Mean no. worms per person	10	17	10	22	19	NG	11
Refs	18	12[c]	15	16	19[d]	17	11[e]

[a] Abbreviations: NA, not applicable; NG, not given.
[b] The fitted line for all studies was a power function of the form $y = ax^b$ in which a and b are constants.
[c] A. Hall, unpublished.
[d] L. Kightlinger, unpublished.
[e] C. Holland, unpublished.

also likely that some of the differences in egg counts shown here resulted from the diagnostic techniques used, or differences in the same technique used by different investigators. For example, a comparison of the ether sedimentation and Kato–Katz techniques for diagnosing *Schistosoma mansoni* found that the sedimentation method detected more infections, but that the egg counts were about a half of those given by the Kato–Katz technique[20]. This could explain the apparently higher fecundity of *A. lumbricoides* in Mexico (Fig. 3a), although it cannot be assumed that the eggs of all worm species behave in the same way in different quantitative techniques. In addition, no technique takes into account variability between people in the dilution of eggs in faeces and the daily fluctuations in egg excretion that have been shown to occur[3], they simply assume that, given a reasonable sample size, differences even out.

Whatever the cause of the differences between countries presented here, the analysis suggests that faecal egg counts might not be very useful when trying to compare the intensity of *A. lumbricoides* infection between countries. There is no reason, however, why they should not still be used to assess the impact of helminth control measures within a single site[21,22]. But this analysis brings into question the use of a threshold of 50 000 epg both to define a heavy infection with *A. lumbricoides* and to decide on the frequency with which mass treatment should be applied – unless, of course, such a threshold was decided locally. We do not recommend a locally decided threshold because it would need to be done in almost every country of the world, and collecting worms from people after anthelmintic treatment is neither simple nor straightforward.

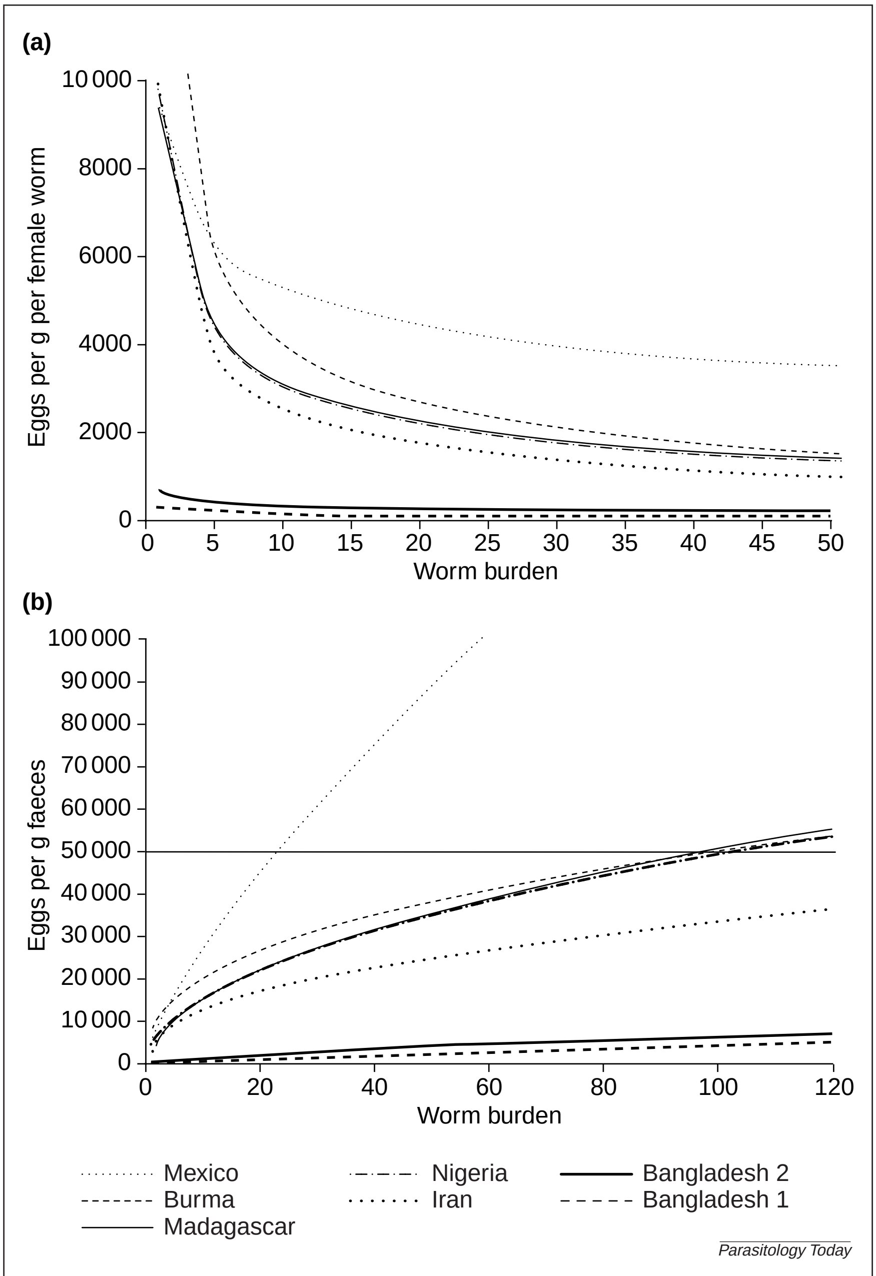

Fig. 3. The relationship between the worm burden and the fecundity of female worms measured as eggs per g faeces (epg) per female worm in seven sites in six countries (a). The lines are derived from the power function $y = ax^b$ and the values of the constants a and b are given in Table 2 with the references for each study. The relationship between worm burden and the concentration of eggs in faeces derived from data presented in (a) in which epg per female worm is multiplied by the total number of worms and then divided by two, assuming an even sex ratio (b).

Periodic mass treatment of helminth infections is, by contrast, simple to do and inexpensive, especially when targeted at groups such as schoolchildren[23,24], but the guidance about when treatments should be applied also needs to be clear and simple. If egg counts are not a universally reliable indicator of the intensity of infection then perhaps the decision to treat more than once a year in the first few years of a control programme, which is undeniably justified in some places, should be based on the initial prevalence of infection, with the aim of keeping the prevalence below the WHO threshold of 50% at which mass treatment is warranted[7]. Although prevalence is a poor indicator of the mean worm burden, when the prevalence is >70% the worm burden, and thus the likelihood of morbidity, increases non-linearly and rapidly[25], and reinfection is typically rapid. The main advantage of prevalence is that it can be determined by a simple direct faecal examination smear. As a microscope is the most basic diagnostic tool in district health laboratories, this enables surveys and decision making to be carried out at the periphery and means that helminth control programmes can be organized by communities. This can only be a good thing.

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References
1 Chan, M-S. (1997) The global burden of intestinal nematode infections – fifty years on. *Parasitol. Today* 13, 438–443
2 de Silva, N.R. *et al.* (1997) Morbidity and mortality due to ascariasis: re-estimation and sensitivity analysis of global numbers at risk. *Trop. Med. Int. Health* 2, 519–528
3 Hall, A. (1982) Intestinal parasites of man: the interpretation of egg counts. *Parasitology* 85, 605–613
4 WHO (1987) *Prevention and Control of Intestinal Parasitic Infections*, WHO/CTD/SIP/98.1 World Health Organization
5 Montresor, A. *et al.* (1998) *Guidelines for the Evaluation of Soil-transmitted Helminthiasis and Schistosomiasis at Community Level. A Guide for Programme Managers*, World Health Organization
6 Anderson, R.M. and May, R.M. (1982) Population dynamics of human helminth infections: control by chemotherapy. *Nature* 297, 557–563
7 WHO (1995) *Health of School Children: Treatment of Intestinal Helminths and Schistosomiasis* (WHO/SCHISTO/95.112; WHO/CDS/95.1), World Health Organization.
8 Hall, A. *et al.* (1992) The intensity of reinfection with *Ascaris lumbricoides* and its implications for parasite control. *Lancet* 339, 1253–1257
9 Albonico, M. *et al.* (1994) A randomised controlled trial comparing mebendazole 500 mg and albendazole 400 mg against *Ascaris, Trichuris* and the hookworms: 4 and 6 months follow up. *Trans. R. Soc. Trop. Med. Hyg.* 89, 538–541
10 Montresor, A. *et al.* (1999) *Monitoring Helminth Control Programmes. Guidelines for monitoring the impact of control programmes aimed at reducing morbidity caused by soil-transmitted helminths and schistosomes, with particular reference to school-age children*. WHO/CDS/CPC/SIP/99.3 World Health Organization
11 Holland, C.V. *et al.* (1989) The epidemiology of *Ascaris lumbricoides* and other soil-transmitted helminths in primary school children from Ile-Ife, Nigeria. *Parasitology* 99, 275–285
12 Hall, A. *et al.* (1999) The distribution of *Ascaris lumbricoides* in human hosts: a study of 1,765 people in Bangladesh. *Trans. R. Soc. Trop. Med. Hyg.* 93, 503–510
13 Hall, A. (1981) Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. *Trans. R. Soc. Trop. Med. Hyg.* 75, 682–687
14 Abdi, Y.A. *et al.* (1995) *Handbook of Drugs for Tropical Parasitic Infections* (2nd edn), Taylor and Francis
15 Thien-Hlaing, *et al.* (1984) Epidemiology and transmission dynamics of *Ascaris lumbricoides* in Okpo village, rural Burma. *Trans. R. Soc. Trop. Med. Hyg.* 78, 497–504
16 Croll, N.A. *et al.* (1982) The population biology and control of *Ascaris lumbricoides* in a rural community in Iran. *Trans. R. Soc. Trop. Med. Hyg.* 76, 187–197
17 Forrester, J.E. and Scott, M.E. (1990) Measurement of *Ascaris lumbricoides* infection intensity and the dynamics of expulsion following treatment with mebendazole. *Parasitology* 100, 303–308
18 Martin, J. *et al.* (1983) The prevalence and intensity of *Ascaris lumbricoides* infections in Moslem children from northern Bangladesh. *Trans. R. Soc. Trop. Med. Hyg.* 77, 702–706
19 Kightlinger, L.K. *et al.* (1995) The epidemiology of *Ascaris lumbricoides, Trichuris trichiura* and hookworm in children in the Ranomafana rainforest, Madagascar. *J. Parasitol.* 81, 159–169
20 Ebrahim, A. *et al.* (1997) Evaluation of the Kato-Katz thick smear and formol ether sedimentation techniques for quantitative diagnosis of *Schistosoma mansoni* infection. *Am. J. Trop. Med. Hyg.* 57, 706–708
21 Bundy, D.A.P. *et al.* (1992) Evaluating measures to control intestinal parasitic infections. *World Health Stat. Q.* 45, 168–179
22 WHO (1996) *Report of the WHO Informal Consultation on the Use of Chemotherapy for the Control of Morbidity due to Soil-transmitted Nematodes in Humans*. Division of Control of Tropical Diseases. WHO/CTD/SIP/96.2 World Health Organization
23 Asaolu, S.O. *et al.* (1991) Community control of *Ascaris lumbricoides in* rural Oyo State, Nigeria: mass, targeted and selective treatment with levamisole. *Parasitology* 103, 291–298
24 Partnership for Child Development (1998) The cost of large-scale school health programmes which deliver anthelmintics to children in Ghana and Tanzania. *Acta Trop.* 73, 183–204
25 Guyatt, H.L. and Bundy, D.A.P. (1991) Estimating prevalence of community morbidity due to intestinal helminths: prevalence of infection as an indicator of the prevalence of disease. *Trans. R. Soc. Trop. Med. Hyg.* 85, 778–782

Pathogenesis of Lymphatic Disease in Bancroftian Filariasis:
A Clinical Perspective

G. Dreyer, J. Norões, J. Figueredo-Silva and W.F. Piessens

The pathogenesis of lymphatic filariasis has been a matter of debate for many decades. Here, Gerusa Dreyer and colleagues propose a dynamic model of bancroftian filariasis, integrating clinical, parasitological, surgical, therapeutic, ultrasonographic and histopathological data. This model has profound implications for filariasis control programs and the management of the individual patient.

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Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* and *Brugia malayi* occurs in individuals of all ages and both sexes, but prevails in those of low socioeconomic level. Early studies recognized lymphangiectasia as a fundamental alteration in the natural history of LF, but had attributed its cause to the downstream obstruction of lymphatic vessels by the adult worms. Furthermore, interstitial fibrosis triggered by diffusible substances released by live or dead worms was thought to have an immunological basis. Traditionally, the natural history of LF has been considered as a wide spectrum of clinical and evolutionary forms in a pre-established sequence – from uninfected individuals at one extreme, through disease-free microfilaria (Mf) carriers, to patients with chronic pathology.

Multidisciplinary studies in bancroftian filariasis

From a multidisciplinary perspective, several problems have hampered studies on the pathogenesis of filarial disease. First, it is difficult to determine whether a given individual is (or was) actively infected, because all existing diagnostic techniques are to some extent

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