

Paradoxical helminthiasis and giardiasis in Cape Town, South Africa: epidemiology and control

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ABSTRACT

Background. South Africa has endorsed a World Health Assembly (WHA) resolution calling for control of soil-transmitted helminths (STHs). In Cape Town, services and housing that exist in old-established suburbs should minimise the prevalence of intestinal parasitic infections, even when residents are poor. Where families live in shacks in densely-populated areas without effective sanitation, more than 90% of children can be infected by STHs. The humoral immune response to worms theoretically favours infection by *Mycobacterium tuberculosis* and HIV.

Objectives. Obtain estimates of gender-, age-, school-related and overall prevalence of helminthiasis and giardiasis in a low-income but well-served community. Assess possible sources of infection. Alert health services to the need for control measures and the threat from protozoal pathogens. Warn that the immune response to intestinal parasites may favour tuberculosis (TB) and HIV/AIDS.

Methods. A cross-sectional study of the prevalence of helminthiasis and giardiasis was carried out in a large, non-selective sample of children attending nine schools. Gender, school and age effects were related to non-medical preventive services, sewage disposal practices and possible sources of infection.

Results. The overall STH infestation rate was 55.8%. Prevalence was influenced by school and age but not by gender. Eggs and cysts were seen at the following prevalences: *Ascaris* 24.8%; *Trichuris* 50.6%; *Hymenolepis nana* 2.2%; *Enterobius* 0.6%; *Giardia* 17.3%; hookworm 0.08%; and *Trichostrongylus* 0.1%. Approximately 60% of sewage sludge is used in a form that will contain viable eggs and cysts.

Conclusions. Prevalence trends in this old community in Cape Town could indicate infection by swallowing eggs or cysts on food or in water, more than by exposure to polluted soil. Sewage sludge and effluent might be sources of infection. In adjacent, under-served, newer communities, promiscuous defaecation occurs. Probable vectors are discussed. The immune response to intestinal parasites might be a risk factor for HIV/AIDS and TB.

Key words: *Ascaris*; *Enterobius*; HIV/AIDS; hookworm; IgE; *Trichuris*; tuberculosis

INTRODUCTION

World Health Assembly (WHA) resolution 54.19 of May 2001 calls for control of soil-transmitted helminthiasis and schistosomiasis, mainly because of the huge burden of subclinical morbidity that these diseases cause in children and women^{1,4}, although worm infestation is also associated with serious clinical disease and mortality^{1,3,5}. WHA member states, including South Africa, were urged to implement regular, non-selective deworming of school-age children and young women by 2010

in areas where the prevalence of worm infestation is 50% or more. As of January 2005, South Africa was still drafting national guidelines for the control of soil-transmitted helminths (STHs) and schistosomiasis, in line with the WHA resolution. Direct responsibility for service delivery to the public lies with provincial health departments in nine provinces. However, participation by the education services as well as non-governmental organisations and the public, private and research sectors, is crucial in order to increase capacity, spread responsibility, generate confidence and monitor progress. In terms of this overall process, no regular, synchronised, sustained, school-based deworming has yet been carried out in South Africa; but treatment is available at health facilities for pre-school children and symptomatic cases.

In the Western Cape province of South Africa, research projects to help resolve practical, operational and legal issues around school-based deworming have made progress⁵⁻⁹. Surveys at 46 primary schools serving old-established, mainly Afrikaans-speaking, urban and rural communities within a 150

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km radius of Cape Town have revealed a median school-based STH prevalence of 41% (range 7%-83%)⁵⁻⁸. In newer, slum-like areas in the city that have been formed by a rapid influx of mainly Xhosa-speaking people from the Eastern Cape province, more than 90% of children can be infested by *Ascaris* and/or *Trichuris*^{5,9}. The existing parasitological background emphasises the need for current information for traditional, mainly Afrikaans-speaking communities in Cape Town where the average income of families is low but services that are required for non-medical prevention of infection by STHs and intestinal protozoa have been in place for a long time (e.g. > 25 years). Based on this need, a cross-sectional study was undertaken at all the primary schools in a traditional area (as defined above).

A general objective was to achieve broad-based estimates of the prevalence of helminthiasis and giardiasis by obtaining faecal samples from a high percentage of children attending all the schools in the community. Specific objectives were to define gender-, age- and school-related prevalences of infection; make a preliminary epidemiological assessment of possible sources of infection and means of transmission; alert health services to the need for control measures and the threat from protozoal pathogens, as necessary; and warn that co-endemic TB¹⁰ and HIV/AIDS^{10,11} may use the immune response to intestinal parasites opportunistically^{2,3,8,12-23}.

METHODS

The study was based on nine primary schools serving a community of ~40 000 people, which is representative of a large proportion of mainly Afrikaans-speaking, traditional inhabitants of Cape Town. The study area lies in the southern part of the suburb of Parow. The community was targeted because although families living there are relatively poor, a comprehensive range of services and good housing have been in place for >25 years. The existing services include clean water, flush toilets, garbage disposal, clinics, electrification and macadamised roads. When the study was undertaken, there were 5607 pupils in the schools, the confirmed TB case rate in the community was 320/100 000, and 5.2% of women attending antenatal clinics were seropositive for HIV¹⁰. The intention was to obtain broad-based estimates of the prevalence of infection by STHs and *Giardia* by obtaining faecal samples from a large majority of the schoolchildren by non-selective sampling. Therefore, all the children were invited to provide faecal specimens. In addition to determination of the effectiveness of existing, non-medical preventive services, this approach was aimed at meeting the specific objectives of analysis of prevalence in relation to gender, school and age and consideration of potential sources of infection, aspects of transmission, and possible explanations for data trends.

Permission to undertake the study was obtained from the Ethics Committee of the South African Medical Research Council, and was approved by the school committees, parents

or guardians. Children participated voluntarily. Each child was given a thread-topped, sterile container identified by a numerical code, in which to place a faecal specimen. School nurses, researchers and parents assisted children. The only reasons for exclusion were a sample that was too small to use or damage to the code label that made it unclear. A standard formol-ether concentration technique was used to process the specimens²⁴. For quality control, two experienced microscopists worked independently to detect the presence of worm eggs and *Giardia* cysts in faecal subsamples. The influence of gender, age and school as independent variables on the presence or absence of eggs and cysts in faeces as dependent binary variables (1 = present and 0 = absent) was determined by logistic analysis of variance (SAS version 9.1). Wald chi-square statistics were used to test for differences in mean prevalence for age and school. Nine discontinuous age classes were based on the number of birthdays elapsed. A probability of less than 5% was interpreted as significant ($p < 0.05$). All children in the schools, including those who did not provide faeces, were dewormed with mebendazole (Vermox®, Janssen-Cilag) after the faecal samples had been collected.

Current sewage processing and disposal methods used in Cape Town were ascertained by means of correspondence with the city's Wastewater Department and discussion with personnel. The city follows national guidelines that were first published in 1997 and amended in 2002²⁵.

RESULTS

A large majority of children provided faecal samples. Overall compliance was 72.6% (4069/5607), with a school-based range of 67.9% -78.3%. The usable samples were from 1935 boys, 1936 girls and 19 individuals for whom gender was not recorded ($n = 3890$), after 179 specimens had been discarded because damage to labels had obscured the code and/or the quantity of faeces was too small. Results for boys and girls were combined because gender did not influence the prevalence of *Ascaris*, *Trichuris* or *Giardia* significantly, whereas age and school did. The data and significant differences ($p < 0.05$) are summarised in Table 1. Concurrence between microscopists was 100% for detection of *Ascaris* and *Trichuris* eggs and exceeded 96% for all other results.

The overall infection rates for STHs and *Giardia*, based on a single faecal sample from each child, were 55.8% (SE 0.9%) and 17.3% (SE 0.6%), respectively. The true prevalence of giardiasis will exceed that detected because the cysts are shed intermittently^{26,27}. School-related prevalence differed for *Ascaris*, *Trichuris* and *Giardia*. In five schools, STH prevalence ranged from 50.0% to 79.6%, which indicates the need for school-based deworming according to WHA criteria. In the other four schools, the STH prevalence range was 34.2%-46.1%. Age-related prevalence of ascariasis did not differ in children between six and 14 years old, whereas that of trichuriasis was higher in those who were 11, 13 and 14 years

Table 1. Mean school- and age-related prevalence (%)* of helminthiasis and giardiasis in children (with standard errors bracketed).

	<i>Asc/Trich</i> eggs [†]	<i>Ascaris</i> eggs	<i>Trichuris</i> eggs	<i>Giardia</i> cysts	Children
Overall	55.8 (0.9)	24.8 (0.7)	50.6 (0.8)	17.3 (0.6)	3890
Schools (n = 9)					
A	40.2 (3.6)*	16.9 (2.2) [‡]	36.5 (3.5)*	10.6 (2.2)*	189
B	59.2 (2.2) [‡]	22.2 (1.7)	55.4 (2.2)**	18.4 (1.7)	495
C	34.2 (2.1)*	8.3 (1.6)*	32.1 (2.1)*	14.7 (1.6)	517
D	37.6 (2.5)*	9.9 (1.4)*	35.5 (2.5)*	8.6 (1.4)*	383
E	67.3 (2.1)**	38.2 (1.8)**	61.8 (2.1)**	22.1 (1.8)**	526
F	78.0 (1.8)**	47.2 (1.8)**	71.2 (2.0)**	21.5 (1.8)**	517
G	46.1 (2.4)*	15.4 (1.9)*	41.5 (2.4)*	18.8 (1.9)	436
H	50.0 (2.2)**	22.1 (1.7)	41.3 (2.2)*	17.9 (1.7)	508
I	79.6 (2.3)**	37.3 (2.7)**	74.6 (2.4)**	16.3 (2.1)	319
Ages in years					
6	43.1 (2.1)*	22.4 (1.8)	36.0 (2.1)*	22.4 (1.8)**	541
7	56.8 (2.3)	26.3 (2.0)	52.8 (2.3)	19.8 (1.8)**	475
8	58.7 (2.3)	27.4 (2.1)	52.9 (2.3)	22.9 (2.0)**	467
9	55.9 (2.3)	23.7 (2.0)	51.5 (2.3)	16.4 (1.7)	476
10	57.4 (2.2)	26.8 (2.0)	50.4 (2.3)	15.6 (1.6)	488
11	59.5 (2.3) [‡]	23.6 (2.0)	56.0 (2.3)**	18.1 (1.8)	454
12	54.1 (2.3)	24.2 (2.0)	49.3 (2.3)	13.6 (1.6)	471
13	59.1 (2.8)	25.3 (2.5)	57.1 (2.9)**	11.3 (1.8)*	301
14+	59.9 (3.3) [‡]	23.5 (2.9)	57.6 (3.4)**	7.4 (1.8)*	217

Low or high ($p < 0.05$) age- or school-related means are indicated by single* or double** asterisk superscripts, respectively.

[†]*Asc/Trich* = specifically the presence of *Ascaris* and/or *Trichuris* eggs.

[‡] $p = 0.05$.

[‡] $p = 0.07$.

[‡] $p = 0.09$.

of age and lower in six-year-olds. There was more giardiasis in six, seven and eight-year-olds, and less in 13- and 14-year-olds. Eggs of the dwarf tapeworm (*Hymenolepis nana*) were present in 2.2% (SE 0.23) of all samples, with a school-range of 0.3%-4.6%. *Enterobius* eggs were seen in 25 faecal specimens, which underestimates prevalence because perianal sampling is necessary^{26,28}. Hookworm-like eggs were detected in three specimens (prevalence 0.08%; SE 0.05), all of which also contained eggs of other STHs. Four children passed *Trichostrongylus*-type eggs in their faeces (prevalence 0.1%; SE 0.05).

Enquiry-based research revealed that in Cape Town, sewage sludge is processed according to national guidelines that do not specify destruction of worm eggs or protozoal cysts as a requirement²⁵. Total sludge production is about 53 000 tons per year on a dry basis. About 32 000 tons (~60%) is partially dried (solids content ~14%) and sold to farmers on condition

that it must be ploughed into the ground for growing cereals or grass but not other crops. This component will contain viable helminth eggs and protozoal cysts. The remainder is either pelletised at temperatures between 120°C and 350°C (~16 000 tons, i.e. 30%), or composted at temperatures exceeding 75°C for five days inside the rows (~5 000 tons, i.e. 9%). Temperatures in the pelletising processes will destroy eggs and cysts, but some could survive composting if the maximum temperature does not reach all parts of the product. More than 90% of the water component of sewage, comprising ~550 megalitres a day, is discharged into rivers as liquid effluent, and will contain helminth eggs and protozoal cysts. The rivers flow into the sea within the city limits, sometimes close to recreational beaches. About 6% of raw sewage is pumped directly into the sea, mostly through long pipelines. The salinity of sea water is such that it should sustain the viability of helminth eggs, and probably protozoal cysts as well.

DISCUSSION

Many communities throughout South Africa do not have the public health services or quality of housing that are available at the study site^{5,8,9}. This reinforces the need to implement WHA resolution 54.19 wherever the prevalence of STHs is high. The resolution does not cover *Enterobius* or *Hymenolepis*, both of which occur in the community studied and elsewhere in the country²⁸. Therefore, control measures should cater for all endemic helminths. Additionally, pathogenic intestinal protozoa can sometimes be an even more serious health threat than worms, both locally and on other continents. In Cape Town, an outbreak of amoebiasis occurred 14 kilometres from the centre of the city in 1984²⁹. In South Milwaukee in the USA, the largest epidemic of waterborne infectious disease ever recorded in a well-developed country affected 403 000 people in 1993. The causative organism was *Cryptosporidium* and the source was leakage of sewage into the water supply³⁰. Infected people developed diarrhoea of varying severity, several immunocompromised individuals died and the overall cost was enormous. The occurrence of *Cryptosporidium* in South Africa has frequently been shown whenever appropriate staining techniques³¹ have been used.

Trends in the STH infection rates at the study site indicate that classical exposure to embryonated eggs in soil through behaviour characteristic of gender and age was probably not the main source of infection. On the contrary, involuntary exposure through contamination of food and possibly drinking water, by STH eggs and *Giardia* cysts appears to be taking place. This would explain why there was no gender difference in prevalence, and why the prevalence of ascariasis did not differ significantly with age. That trichuriasis was significantly more prevalent in older children is not contradictory because the adult worms live in the host for about three years²⁶. This has a cumulative effect through new infections imposed on those already present. *Ascaris* adults have a much shorter lifespan of about one year²⁶. The significantly higher prevalence of giardiasis in younger children may be the result of resistance developing with ongoing exposure^{26,27}. In Cape Town, there is clearly a need for in-depth analysis and monitoring of sources of infection by intestinal pathogens because a large proportion of the city's population lives under conditions similar to those at the study site, or at greater exposure to infection where there is overcrowding and promiscuous defaecation⁹.

The national guidelines on recycling of sewage are intended to ensure utilisation of the sludge as an organic fertiliser and re-use of the water after purification because both components are valuable resources²⁵. Since the use of products that will contain embryonated STH eggs is allowed, they could be a source of helminthic and protozoal infections if utilised for growing vegetables or fruit to be eaten raw. It is important to obtain comparative data by conducting parasitological surveys

in affluent suburbs of Cape Town where the prevalence of intestinal helminths and protozoa is not known. The results will show whether regular treatment of the entire population of the city with effective anthelmintics is necessary, as well as general implementation of non-medical control measures that are affordable and sustainable. Mechanical transfer of eggs and cysts from human faeces or sewage to food and/or water could be happening on a significant scale. It is generally known that flies, cockroaches and rats are problems in the city. Flies are ubiquitous, especially in summer, and are likely to be vectors.³²⁻³⁵ Rats in Cape Town could be reservoir hosts of the dwarf tapeworm *Hymenolepis nana* and may host strains of *Giardia* that infect humans²⁶. There are also many dogs in the city. They can be important disseminators of some STH eggs and protozoa but are probably not a significant risk in respect of zoonotic protozoal pathogens³⁷. Strong winds are a feature of the climate in the south-western Cape and may blow embryonated STH eggs about as a component of dust, as has been reported from Japan³⁶. Eggs transferred by wind could originate either from human faeces in places in the city where people defaecate on the ground because they cannot access functional toilets⁹, or from sewage products used as fertiliser.

The detection of hookworm-like eggs and *Enterobius* eggs during this study are both of importance, for different reasons. The Mediterranean-type climate in the south-western Cape may be highly suitable environmentally for *Ancylostoma duodenale* if it were to be introduced by migrants or visitors from places where it is endemic. This threat needs to be monitored because hookworm can be a major cause of anaemia, especially in pregnant women^{1,3,4}. *Enterobius vermicularis* (pinworm) is not a soil-transmitted helminth and is particularly important within families, especially affluent ones²⁶. This is partly because the light eggs are easily disseminated by air movement within the household. Familial stress because of irritability caused by sleep disturbance secondary to perianal pruritus is characteristic of enterobiasis^{26,28}.

In terms of the criteria agreed under WHA resolution 54.19, it can be concluded that non-selective, regular, synchronised deworming should be implemented in five of the nine schools in the study area. However, even when the prevalence of worm infestation is less than 50%, it can be recommended that regular deworming should probably be implemented, based on the possibility that HIV/AIDS and TB might use the dominant humoral immune response to helminthiasis opportunistically in terms of faster infection dynamics and disease progression. This kind of immune profile may also impair vaccination^{2,3,8,12-23}. There is strong serological evidence from the study site for this reasoning. New research has revealed that 15% of a random sample of children ($n = 359$) had *Ascaris* eggs in their faeces but 48% had elevated *Ascaris*-specific IgE in serum (C.C. Obihara, unpublished data). Elevated *Ascaris*-specific IgE

indicates a competent immune response to either larval stages or adult worms. After destruction of the original parasites, it is likely to be boosted by a new challenge when *Ascaris* is endemic. Hence, Ascaris-specific IgE it is inevitably more frequent than eggs in faeces, and egg-positivity may indicate immunological incompetence in some individuals^{21,23}. Regular, non-selective deworming could minimise humoral immune dominance and thereby reduce the threat of HIV/AIDS and TB. This kind of effect should be particularly important with regard to immunisation against HIV before sexual activity starts^{11,22,23}. In poorer, more recently formed city communities and in rural areas, the need is greater because between 70% and >90% of school-age children in the south-western Cape have been found to be infected by worms⁵⁻⁹, and giardiasis is widespread^{6,7,27}. Potential vaccines that are intended to elicit a cellular immune response against HIV may be particularly vulnerable to reduction in efficacy when helminthiasis is highly endemic^{22,23}, especially when used prepubertally¹¹. Like helminths, intestinal protozoa can elicit a predominantly humoral immune response²⁶.

Control of worm infestation in southern Africa as advocated by the WHA¹⁻⁴, should not be delayed pending assessment of the extent to which helminthiasis may interact with various co-endemic diseases, or whether regular deworming may modulate the amount of allergy in communities^{38,39}. Adjustments in policy and practice can be made when necessary, based on monitoring of results and new research findings.

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REFERENCES

1. **Crompton DWT, Montresor A, Nesheim MC, Savioli L** (eds). Controlling disease due to helminth infections. World Health Organization, Geneva, 2003.
2. **Colley DG, Secor WE**. Immunoregulation and World Health Assembly resolution 54.19: why does treatment control morbidity? *Parasitol. Int.* 2004; 53:143-50.
3. **Editorial**. Thinking beyond deworming. *Lancet* 2004; 364:1993-4.
4. **Savioli L, Albonico M, Engels D, Montresor A**. Progress in the prevention and control of schistosomiasis and soil-transmitted helminths. *Parasitol. Int.* 2004; 53:103-13.
5. **Adams VJ, Lombard CJ, Dhansay MA, Markus MB, Fincham JE**. Efficacy of albendazole against the whipworm *Trichuris trichiura* - a randomised, controlled trial. *S. Afr. Med. J.* 2004; 94:972-6.
6. **Arendse VJ**. Treatment and prevention of trichuriasis: efficacy of albendazole in disadvantaged children at Rawsonville Primary School, Western Cape Province, South Africa. 2001. MScMedSc thesis, University of Stellenbosch, South Africa.
7. **Fincham JE, Adams V, Curtis B, Jordaan E, Dhansay MA**. Intestinal parasites, growth of children, sanitation and water quality at primary schools in the Boland/Overberg health region, Western Cape Province. South African Medical Research Council, Cape Town, 2001. ISBN 1-919809-21-X.
8. **Fincham JE, Markus MB, Adams VJ, et al**. Association of deworming with reduced eosinophilia: implications for HIV/AIDS and co-endemic diseases. *S. Afr. J. Sci.* 2003; 99:182-4.
9. **Anonymous**. The Khayelitsha Task Team - building health partnerships that work. South African Medical Research Council, Cape Town, 2001. ISBN 1-919809-26-0.
10. **Verver S, Warren RM, Munch Z, et al**. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet* 2004; 363:212-4.
11. **Makgoba MW, Solomon N, Tucker TJP**. The search for an HIV vaccine. *Brit. Med. J.* 2002; 324:211-3.
12. **Markus MB, Fincham JE**. Worms and pediatric human immunodeficiency virus infection and tuberculosis. *J. Infect. Dis.* 2000; 181:1873.
13. **Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S**. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guérin (BCG) vaccination. *Clin. Exp. Immunol.* 2001; 123:219-25.
14. **Markus MB, Fincham JE**. Helminthiasis and HIV vaccine efficacy. *Lancet* 2001; 357:1799.
15. **Markus MB, Fincham JE**. Helminthic infection and HIV vaccine trials. *Science* 2001; 291:46-7.
16. **Markus MB, Fincham JE**. Implications for neonatal HIV/AIDS and TB of sensitization *in utero* to helminths. *Trends Parasitol.* 2001; 17:8.
17. **Fincham JE, Markus MB, Brombacher F**. Vaccination against helminths: influence on HIV/AIDS and TB. *Trends Parasitol.* 2002; 18:385-6.
18. **MacDonald AS, Araujo MI, Pearce EJ**. Immunology of parasitic helminth infections. *Infect. Immun.* 2002; 70:427-33.
19. **Trist o-S R, Ribeiro-Rodrigues R, Johnson LT, Pereira FEL, Dietze R**. Intestinal nematodes and pulmonary tuberculosis. *Rev. Soc. Brasil. Med. Trop.* 2002; 35:533-5.
20. **Elliott AM, Kyosiimire J, Quigley MA, et al**. Eosinophilia and progression to active tuberculosis in HIV-1-infected Ugandans. *Trans. R. Soc. Trop. Med. Hyg.* 2003; 97:477-80.
21. **Fincham JE, Markus MB, Adams VJ**. Could control of soil-transmitted helminthic infection influence the HIV/AIDS pandemic? *Acta Trop.* 2003; 86:315-33.

22. **Robinson TM, Nelson RG, Boyer JD.** Parasitic infection and the polarized Th2 immune response can alter a vaccine-induced immune response. *DNA Cell Biol.* 2003; 22:421-30.
23. **Borkow G, Bentwich Z.** Chronic immune activation associated with chronic helminthic and human immunodeficiency virus infections: role of hyporesponsiveness and anergy. *Clin. Microbiol. Rev.* 2004; 17:1012-30.
24. **Ash LR, Orihel TC.** Parasites: a guide to laboratory procedures and identification. American Society of Clinical Pathologists, 1987. ASCP Press, Chicago, p 23.
25. **Anonymous.** Permissible utilisation and disposal of sewage sludge. Edition 1, addendum 1. Water Research Commission, Department of Water Affairs and Forestry, Department of Agriculture, and Department of Health, South Africa. TT 85/97, August 1997 and TT 154/01, July 2002.
26. **Roberts LS, Janovy J.** Foundations of parasitology, 6th ed. Singapore: McGraw-Hill, 2000.
27. **Kwitshana ZL.** *In vitro* culture and isoenzyme electrophoresis of *Giardia lamblia*. 2000. MSc thesis, University of Natal, South Africa.
28. **Mosala TI, Appleton CC.** True prevalence of the pinworm (*Enterobius vermicularis*) among children in Qwa-Qwa, South Africa. *S. Afr. J. Sci.* 2003; 99:465-6.
29. **Whittaker S, Jackson TFHG, Gathiram V, Regensberg LD.** Control of an amoebiasis outbreak in the Phillippi area near Cape Town. *S. Afr. Med. J.* 1994; 84:389-93.
30. **Guerrant RL.** Cryptosporidiosis: an emerging, highly infectious threat. *Emerg. Infect. Dis.* 1997; 3:51-7.
31. **Markus MB, Bush JB.** Staining of coccidial oocysts. *Vet. Rec.* 1987; 121:329.
32. **Sulaiman S, Sohadi AR, Yunus H, Ibrahlim R.** The role of some cyclorrhaphan flies as carriers of human helminths in Malaysia. *Med. Vet. Entomol.* 1988; 2:1-6.
33. **Monzon RB, Sanchez AR, Tadiaman BM, et al.** A comparison of the role of *Musca domestica* (Linnaeus) and *Chrysomya megacephala* (Fabricius) as mechanical vectors of helminthic parasites in a typical slum area of metropolitan Manila. *SE Asian J. Trop. Med. Public Health* 1991; 22:222-8.
34. **Markus MB.** Tuberculosis and HIV infection. *Lancet* 1993; 342:677.
35. **Szostakowska B, Kruminis-Lozowska W, Racewicz M, et al.** *Cryptosporidium parvum* and *Giardia lamblia* recovered from flies on a cattle farm and in a landfill. *Appl. Environ. Microbiol.* 2004; 70:3742-4.
36. **Kunii C.** It all started from worms. The 45-year record of Japan's post-World War II national health and family planning movement. Tokyo: The Hoken Kaikan Foundation, 1992.
37. **Traub RJ, Robertson ID, Irwin PJ, Mencke N, Thompson RCA.** Canine gastrointestinal parasitic zoonoses in India. *Trends Parasitol.* 2005; 21:42-8.
38. **Markus MB.** Public health and vaccines - immune responses in developed versus poor countries. *S. Afr. Med. J.* 2003; 93:834-5.
39. **van den Biggelaar AHJ, Rodrigues LC, van Ree R, et al.** Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J. Infect. Dis.* 2004; 189:892-900.