Treatment with anthelminthics during pregnancy: what gains and what risks for the mother and child?

ALISON M. ELLIOTT[1,2], JULIET NDIBAZZA[1], HARRIET MPAIRWE[1], LAWRENCE MUHANGI[1], EMILY L. WEBB[2], DENNISON KIZITO[1], PATRICE MAWA[1], ROBERT TWEYONGYERE[3] *and* MOSES MUWANGA[4] for the ENTEBBE MOTHER AND BABY STUDY TEAM

[1] *MRC/UVRI Uganda Research Unit on AIDS, Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda*
[2] *London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK*
[3] *Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda*
[4] *Entebbe Hospital, PO Box 29, Entebbe, Uganda*

SUMMARY

In 1994 and 2002, respectively, the World Health Organisation proposed that treatment for hookworm and schistosomiasis could be provided during pregnancy. It was hoped that this might have benefits for maternal anaemia, fetal growth and perinatal mortality; a beneficial effect on the infant response to immunisation was also hypothesised. Three trials have now been conducted. Two have examined the effects of benzimidazoles; one (the Entebbe Mother and Baby Study) the effects of albendazole and praziquantel. All three were conducted in settings of high prevalence but low intensity helminth infection. Results suggest that, in such settings and given adequate provision of haematinics, the benefit of routine anthelminthics during pregnancy for maternal anaemia may be small; none of the other expected benefits has yet been demonstrated. The Entebbe Mother and Baby Study found a significant adverse effect of albendazole on the incidence of infantile eczema in the whole study population, and of praziquantel on the incidence of eczema among infants of mothers with *Schistosoma mansoni*. Further studies are required in settings that differ in helminth species and infection intensities. Further research is required to determine whether increased rates of infantile eczema translate to long-term susceptibility to allergy, and to explore the underlying mechanisms of these effects. The risks and benefits of routine anthelminthic treatment in antenatal clinics may need to be reconsidered.

Key words: Anthelminthic, pregnancy, albendazole, praziquantel, hookworm, *Schistosoma mansoni*, atopic eczema, anaemia.

INTRODUCTION

Hookworm causes iron deficiency and anaemia (Roche and Layrisse, 1966); and maternal iron deficiency anaemia is associated with adverse pregnancy outcomes including still birth, prematurity, low birth weight and possibly maternal mortality (Allen, 2000). Because of this, in 1994, an informal consultation of the World Health Organisation (WHO) recommended that hookworm control using levamisole or pyrantel, or the benzimidazoles albendazole or mebendazole, should be included in strategies for the improvement of health in girls and women in areas where hookworm is endemic and anaemia prevalent (WHO, 1995). In particular it was suggested that a single dose of anthelminthic treatment could be given in pregnancy, during the second to third trimester; routine anthelminthic treatment was already being implemented during antenatal care in Sri Lanka (Atukorala *et al*. 1994; de Silva *et al*.

1999). However, at that time no randomised trials to assess the risks and benefits of this intervention had been undertaken.

A further consultation in 2002 considered the use of praziquantel during pregnancy and lactation (WHO, 2002); previous recommendations had been against this. The consultation recognised that the former recommendations could result in exclusion of women in endemic areas from treatment of schistosomiasis for a large proportion of their reproductive lives. It was proposed that as well as specific end-organ damage, schistosomiasis in pregnancy might contribute to important subtle maternal morbidity including anaemia and decreased work capacity, and perhaps to impaired fetal growth. There was no evidence of fetal toxicity for praziquantel in animals, and its use during human pregnancy in a small number of documented cases had shown no severe adverse effects. Therefore the consultation recommended the use of praziquantel during pregnancy. Again, no randomised trials of praziquantel treatment during pregnancy had been undertaken and in 2006 a WHO working group on schistosomiasis listed among its priorities to "conduct without delay randomised, placebo-controlled trials of praziquantel

Corresponding author: Alison M. Elliott, MRC/UVRI Uganda Research Unit on AIDS, P.O. Box 49, Entebbe, Uganda. Tel: +256 752 720609; Fax: +256 414 321137; E-mail: alison.tom@infocom.co.ug

treatment in pregnancy for all species of human schistosome in areas of both high and low transmission" (WHO, 2006).

Since these meetings, two randomised trials on the use of benzimidazoles during pregnancy have been reported (Haider *et al*. 2009), one from Peru (Larocque *et al*. 2005, 2006) and one from Sierra Leone (Torlesse and Hodges, 2001), and in Uganda we have conducted a randomised trial of both albendazole and praziquantel during pregnancy, using a 2×2 factorial design: the Entebbe Mother and Baby Study (Elliott *et al*. 2007). We here summarise the results of our trial, with follow-up of infants to one year of age, and discuss the implications of our findings, and of the findings of other recent studies, for recommendations on the routine use of anthelminthic drugs during pregnancy, and for further research priorities.

The Entebbe Mother and Baby Study [ISRCTN32849447]

Although the WHO consultations focused mainly on potential benefits for anaemia and helminth-specific pathology in the mother and for growth in the fetus, there are other important potential effects of helminths and anthelminthic treatment during pregnancy. Helminths have a major impact on the host immune system, modulating the response both to themselves and to unrelated antigens (van Riet *et al*. 2007). Before the start of our trial, it had been observed that exposure to maternal helminths *in utero* could result in sensitisation of the fetus to helminth antigens (Novato-Silva *et al*. 1992; Malhotra *et al*. 1997), and in modulation of the infant immune response to neonatal BCG immunisation (Malhotra *et al*. 1999). The Entebbe Mother and Baby Study was therefore designed to address the hypothesis that helminth infections can influence the immune response to non-worm antigens (including immunogens and pathogens), that these effects can be established *in utero* and that they can be modified by anthelminthic treatment during pregnancy.

The study commenced in 2002 as a trial of albendazole versus placebo during pregnancy, but was suspended following publication of the WHO recommendations on use of praziquantel during pregnancy (Allen *et al*. 2002) and modified to allow us to investigate effects of praziquantel treatment during pregnancy, as well as of albendazole treatment. Data collected before this modification created a preliminary study of the effects of albendazole treatment during pregnancy among 104 mothers and their infants. In this preliminary study we observed a protective effect of maternal helminth infection, and a possible detrimental effect of albendazole treatment during pregnancy, on the incidence of infantile eczema (Elliott *et al*. 2005). Therefore we also modified the trial design to allow us to investigate

effects of anthelminthics during pregnancy on allergic disease outcomes.

The main Entebbe Mother and Baby Study thus compared single-dose albendazole (400 mg) versus placebo and praziquantel (40 mg/kg) versus placebo, given during the second or third trimester of pregnancy, in a 2×2 factorial design. All women were treated with both albendazole and praziquantel after delivery. The study included a further randomisation of the children, from age 15 months to five years, to quarterly albendazole versus placebo; this part of the study will be completed in 2011 and will not be discussed in this review.

The study is based in Entebbe and the neighbouring sub-county of Katabi, a peninsula in Lake Victoria, Uganda. We recruited 2507 women at the government district hospital between 2003 and 2005. Most women were poor: 82% reported a monthly cash income of less than 30,000 Uganda shillings (then approximately US$20) per month. They came from the relatively urban setting of Entebbe Municipality and from the surrounding fishing communities and rural farming communities. The prevalence of helminth infection at enrolment was high: 68% had at least one helminth infection, 45% had hookworm, 21% *M. perstans* and 18% had *Schistosoma mansoni*; other species were less common (Woodburn *et al*. 2009). For intestinal helminths these results were based on examination of two slides from a single stool sample, implying that the true prevalence of helminths was higher (Hall, 1981; Utzinger *et al*. 2001). Although the prevalence of infection was high, intensity was generally low, according to WHO classifications (WHO, 1995, 1999): 85% of those with hookworm had egg counts below 1000 eggs per gram (epg) of stool (91% below 2000 epg), and 65% of those with *S. mansoni* had counts below 100 epg. At age one year, only 44 of 1358 infants examined had helminth infections, the commonest being *Ascaris* (15 infants), *Trichuris* (12 infants) and *Mansonella* (eight infants); because these numbers were so small the effects of infants' helminth infections are not considered further in this review.

EFFECTS OF ANTHELMINTHIC TREATMENT IN PREGNANCY

On helminth prevalence

Anthelminthic treatment during pregnancy was effective in our study (Ndibazza *et al*. 2010) (Fig. 1). The prevalence of hookworm declined in the albendazole treatment group from 45% to 5%. This was similar to the effect of albendazole reported by Torlesse and Hodges, (2001) but greater than the effect reported by Larocque *et al*. (2006) for single-dose mebendazole, in accord with the greater efficacy of albendazole against hookworm (Keiser and Utzinger, 2008). There was no change in hookworm prevalence in
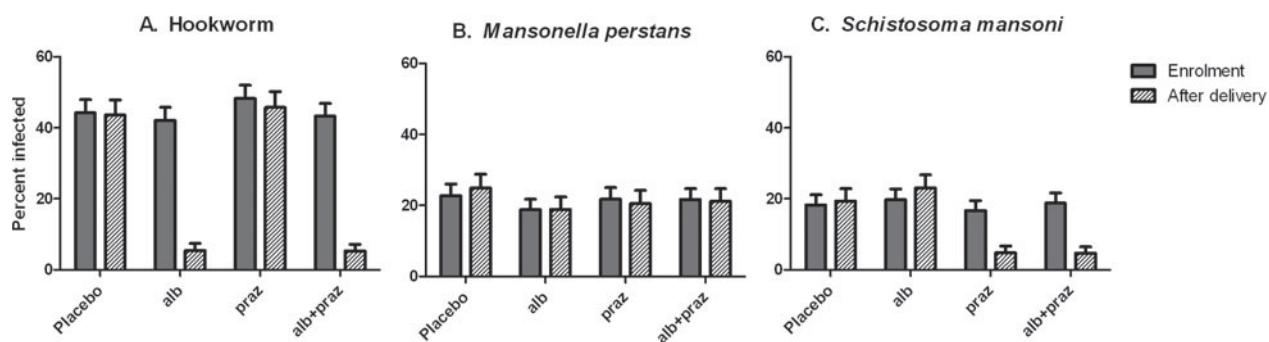
Fig. 1. Prevalence of the three commonest helminth infections at enrolment and after delivery, according to treatment allocation during pregnancy, among 2507 women participating in the Entebbe Mother and Baby Study. Placebo: double placebo group; alb: single dose albendazole (400 mg) plus placebo for praziquantel; praz: single dose praziquantel (40 mg/kg) plus placebo for albendazole; alb + praz: single dose albendazole (400 mg) plus praziquantel (40 mg/kg). Error bars show the upper 95% confidence interval for the percentage prevalence of infection.

the albendazole placebo group. Single-dose albendazole was also effective for *Ascaris*, but had little effect on *Strongyloides* or *Trichuris*. Praziquantel was effective against *S. mansoni*: prevalence declined from 18% to 5%. This is important because pregnancy is associated with immunosuppression and the efficacy of praziquantel may depend partly on immunologically-mediated killing following disruption of the tegument and exposure of antigens on the surface of the parasite (Doenhoff *et al.* 2008). We found that boosts in cytokine and antibody response to schistosome antigens after treatment tended to be lower when praziquantel was given during pregnancy than when it was given after delivery, but the cure rate for *S. mansoni* was similar (Tweyongyere *et al.* 2008, 2009).

On maternal anaemia

The WHO consultations discussed above anticipated that reductions in anaemia would be an important benefit of intervention against hookworm during pregnancy, and also a benefit of treating schistosomiasis during pregnancy. In our study, the prevalence of anaemia at baseline was 40%. No individual helminth species was associated with the presence of anaemia, but increasing intensity of hookworm was associated with lower haemoglobin level (Muhangi *et al.* 2007). These findings were similar to those of Larocque *et al.* (2005) in Peru, where the prevalence and intensity of hookworm infection were similar. By contrast to a study in Tanzania (Ajanga *et al.* 2006), we found no association at baseline between maternal anaemia and intensity of *S. mansoni*.

We provided haematinics routinely to all women, as well as two doses of intermittent presumptive treatment for malaria with sulphadoxine-pyrimethamine. We found no overall benefit of anthelminthic treatment during pregnancy on maternal anaemia after delivery and no benefit of albendazole in the subgroup of mothers shown to have hookworm at

baseline, or of praziquantel among mothers shown to have *S. mansoni* infection (Ndibazza *et al.* 2010). An exploratory analysis stratified for hookworm intensity suggested a possible benefit of albendazole among women with moderate to heavy hookworm, but this difference in effect was not statistically significant. Again, the findings of Larocque *et al.* (2006) were similar: no benefit for maternal anaemia in the context of the provision of haematinics. The Sierra Leone trial (Torlesse and Hodges, 2001) compared single-dose albendazole with haematinics in a factorial design and found some benefit of albendazole but a greater benefit of haematinics for both anaemia and iron status. Together, these results suggest that, given adequate provision of haematinics, the benefit of routine anthelminthics during pregnancy for maternal anaemia may be less than had been anticipated.

On birth weight

The possibility that birth weight would be improved by routine treatment with benzimidazoles during pregnancy in areas of high hookworm prevalence was suggested by the cross-sectional study in Sri Lanka (de Silva *et al.* 1999). Very low birth weight (below 1·5 kg) was less common among women who reported taking mebendazole during pregnancy than among those that did not. Similarly, a non-randomised study of albendazole treatment in Nepal suggested a benefit for birth weight (Christian *et al.* 2004). The principal limitation of these studies was the possibility that taking anthelminthics was associated with better overall care-seeking behaviour and hence better outcomes mediated by a variety of factors. More recently, studies in animals suggested possible adverse effects of schistosomiasis on birth weight and other perinatal outcomes, but no adequate studies have been conducted to explore similar effects in humans (Friedman *et al.* 2007).

Neither we nor Larocque *et al.* (2006) found any benefit of anthelminthic treatment during pregnancy

for mean birth weight or low birth weight (below 2·5 kg) (Ndibazza *et al*. 2010). Larocque and colleagues found a possible benefit of mebendazole for very low birth weight (below 1·5 kg), but only seven infants fell into this category in their study. In our study, 11 infants were very low birth weight and there was no association between this outcome and the treatment the mother had received. Again, the provision of adequate haematinics could be a factor in preventing an adverse effect of hookworm mediated by iron deficiency, and a consequent benefit of albendazole, from becoming evident, but the role of iron and folic acid supplementation in determining pregnancy outcomes other than anaemia remains uncertain (Pena-Rosas and Viteri, 2006).

On congenital anomalies

The principal mode of action of benzimidazoles is to bind tubulin and inhibit the synthesis of microtubules which are ubiquitous components of the eukaryotic cytoskeleton. Their specificity for helminths is relative and selectivity seems to be related to the stability of binding between the drug and the tubulin molecules (Lacey, 1990; MacDonald *et al*. 2004). Thus interference with processes such as mitosis provide a plausible mechanism of fetal toxicity for benzimidazoles, and there is evidence of toxicity in animal models (Dayan, 2003). Although this was recognised by the WHO committee in 1995, differences in dose, metabolism and pharmacokinetics between animal models and human usage led them to suggest that benzimidazoles were probably safe for pregnant and lactating women and their offspring (WHO, 1995). In the cross-sectional study in Sri Lanka, the number of congenital anomalies was higher among infants of women who reported taking mebendazole during the first trimester than among those that did not, but this difference was not statistically significant (de Silva *et al*. 1999) and, overall, observational studies have not reported a significant excess of congenital anomalies following use of benzimidazoles in human pregnancy (Diav-Citrin *et al*. 2003).

The mode of action of praziquantel is less certain (Doenhoff *et al*. 2008), but for this drug there is no evidence of genotoxicity or fetal toxicity (Dayan, 2003; Adam *et al*. 2005).

We avoided anthelminthic treatment during the first trimester, and found no evidence of association between either albendazole or praziquantel and congenital anomalies in our study (Ndibazza *et al*. 2010). Similarly, the trials in Peru and Sierra Leone found no evidence of fetal toxicity for benzimidazoles given in the second trimester (Torlesse and Hodges, 2001; Larocque *et al*. 2006). These findings are encouraging, but pharmacovigilance remains appropriate, especially if anthelminthics are given in the first trimester.

On perinatal and infant mortality

The observational studies in Sri Lanka and Nepal suggested a benefit of benzimidazoles during pregnancy for perinatal mortality and infant survival to 6 months of age (de Silva *et al*. 1999; Christian *et al*. 2004). However, we found no benefit of either albendazole or praziquantel during pregnancy for rates of still birth, neonatal mortality or infant mortality (Ndibazza *et al*. 2010; Webb *et al*. 2011). Similarly, Larocque *et al*. (2006) found no effect of mebendazole on perinatal mortality.

On infant response to immunisation

The evidence that led to the hypothesis that antenatal exposure to helminth infections has an important influence on the infant response to immunisation in low-income and tropical settings has been reviewed elsewhere (Labeaud *et al*. 2009). As discussed above, this hypothesis was the principal motivation for our study. We used a six-day whole blood culture assay at age one year to assess the cellular response to BCG and tetanus immunisation, stimulated using crude culture filtrate proteins of *Mycobacterium tuberculosis* and tetanus toxoid (TT), respectively; we examined production of type 1 (interferon (IFN)-γ) type 2 (interleukin (IL)-5 and IL-3) and regulatory (IL-10) cytokines in supernatant using enzyme-linked immunosorbent assays (ELISAs). ELISAs were also used to assess antibody responses to TT and measles immunisation.

We found no overall effect of either treatment on the response to immunisation (Webb *et al*. 2011). In sub-group analyses we found a reduction in type 2 cytokine responses to tetanus toxoid among infants of mothers with hookworm who received albendazole, compared to those who received placebo, but there was no effect on total immunoglobulin (Ig)G production, the key parameter for protection following tetanus immunisation (Plotkin, 2010). No other effects were observed for the response to BCG, tetanus or measles immunisation in the planned subgroup analyses for effects of albendazole in mothers with hookworm, or of praziquantel in mothers with *S. mansoni*. Such results could occur if effects of helminth exposure were established prior to the trial intervention, say during the first trimester, and could not be reversed by subsequent treatment. This explanation is unlikely since, in observational analyses, infant response to vaccines showed no consistent associations with the presence of maternal hookworm or *S. mansoni* (Elliott *et al*. 2010).

In observational analyses, maternal infection with the filarial helminth, *Mansonella perstans*, was associated with increased IL-10 responses to the vaccine antigens. Curiously, this effect was evident principally in the albendazole placebo group, although *Mansonella* prevalence was not affected by the single

dose of albendazole given. Maternal *Mansonella* showed no effect on type 1 or type 2 responses (Elliott *et al*. 2010).

Taken together, our results suggest that maternal helminth infection is unlikely to be a major contributor to poor efficacy of immunisation in infancy in the tropics, and routine anthelminthic treatment during pregnancy is unlikely to lead to important improvements in this outcome.

On infectious disease incidence in infancy

There is considerable evidence, both from animal models and from studies in humans, that exposure to helminths *in utero* programmes the infant response to subsequent challenge, influencing susceptibility to infection, and immunologically-mediated pathology (Lammie *et al*. 1991; Steel *et al*. 1994; Malhotra *et al*. 2006). Pursuing the idea that some of the immunoregulatory effects of helminths may influence responses to bystander antigens, we investigated whether anthelminthic treatment during pregnancy influenced infant susceptibility to infectious diseases. We found no effect on incidence of malaria or on malaria infection at age one year; and no effect on incidence of diarrhoea or lower respiratory tract infection (Webb *et al*. 2011).

In two previous studies concerning helminths and HIV infection, we had noted a temporary increase in HIV load following treatment of *S. mansoni* (Elliott *et al*. 2003; Brown *et al*. 2005), so a particular concern was that treatment with praziquantel during pregnancy might result in increased intrauterine HIV transmission. Our study had little power to assess this outcome and was inconclusive, but vertical HIV transmission was, if anything, lower among HIV-positive mothers that received praziquantel (OR 0·60 (95% CI 0·29–1·23)) (Webb *et al*. 2011).

On allergy

There is considerable evidence from animal models, and some from studies in humans, that helminth infection can protect against allergic disease through active immunoregulatory pathways (Smits *et al*. 2010). The results of epidemiological studies to date have been less clear-cut. In cross-sectional studies atopy (represented by skin-prick test positivity) has shown a fairly consistent inverse association with helminth infection, and hookworm has shown a consistent inverse association with asthma, but associations with asthma for other helminths, and associations between helminths and eczema, have been variable (Leonardi-Bee *et al*. 2006; Flohr *et al*. 2009). Some, but not all, intervention studies have shown an effect of anthelminthic treatment on skin-prick test responses to allergens but, prior to our study, none had shown an effect on an allergic disease outcome (Lynch *et al*. 1993; van den Biggelaar *et al*. 2004; Cooper *et al*. 2006; Endara *et al*. 2010; Flohr *et al*. 2010).

In the Entebbe Mother and Baby Study, eczema was the commonest allergic condition observed during infancy, as expected for the age group, with an incidence of 10 per 100 person years of follow-up. In a sub-group of infants for whom skin-prick testing was conducted, eczema was strongly associated with skin prick test positivity, indicating that it was atopic. We found that albendazole treatment during pregnancy was associated with an increased risk of eczema in the whole study population (Cox proportional Hazard Ratio (HR) 1·82 (95% CI 1·26–2·64)), while praziquantel treatment was associated with increased risk of eczema among infants of mothers with *S. mansoni* (HR 2·65 (95% CI 1·16–6·08), interaction *P* value 0·02) (Mpairwe *et al*. 2011) (Fig. 2).

For albendazole, we expected that the effects would be strongest among infants of women with hookworm – the commonest species, and most susceptible to albendazole treatment – but, surprisingly, stratification by maternal hookworm status showed no difference in effect (interaction *P* value 0·52). Indeed, an effect of albendazole was seen even among infants of mothers with no detected helminth infection. This may mean that the effect of albendazole was mediated (a) by the drug itself, (b) by an effect on low intensity hookworm, or other albendazole-susceptible helminth species, not detected by the single stool sample examined or (c) by an effect on another organism. Albendazole has a broad spectrum of action, sometimes overlooked in relation to anthelminthic treatment programmes, and candidates for option (c) include malaria, intestinal protozoa, microsporidia and commensal yeasts (Cruz and Edlind, 1997; Skinner-Adams *et al*. 1997; MacDonald *et al*. 2004; Solaymani-Mohammadi *et al*. 2010).

By contrast, our findings for schistosomiasis are entirely consistent with a protective effect of exposure to maternal *S. mansoni* infection that is removed by praziquantel treatment.

Our study is thus the first to demonstrate an effect of anthelminthic treatment on an allergic disease outcome and provides support for the hypothesis that helminths protect against allergic disease. Why did we observe an effect, while previous studies in school-age children did not? Given the low prevalence of allergic disease in rural tropical environments, several of the previous studies had limited power to show an effect on disease outcomes. However, an important possibility is that our study showed an effect because susceptibility to allergic disease is programmed *in utero* or very early in postnatal life, with relatively little effect of subsequent intervention (von Mutius and Le Souef, 2007). Thus, from the perspective of allergology, our results provide important evidence that pre-natal or very
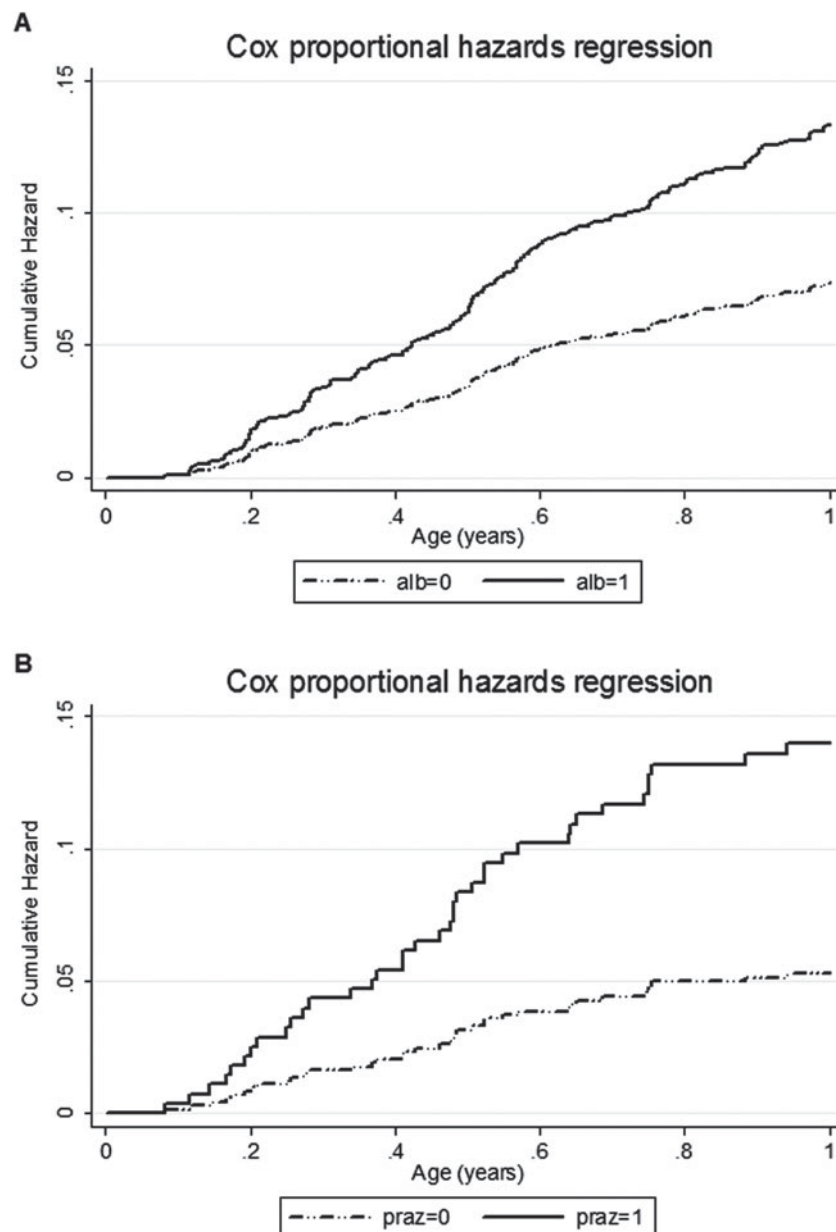
Fig. 2. The effect of treatment with albendazole or praziquantel during pregnancy on the incidence of eczema during infancy in the Entebbe Mother and Baby Study. (A) The effect of albendazole among 2345 live-born infants of all mothers, with or without detected helminth infection. (B) The effect of praziquantel among 421 infants of mothers with detected *Schistosoma mansoni* infection. Shown are the cumulative hazard functions based on multiple eczema events. Alb, albendazole; praz, praziquantel.

early post-natal interventions may allow the primary prevention of allergic disease in children at risk.

CONCLUSIONS AND FURTHER RESEARCH NEEDS

The Entebbe Mother and Baby Study is the first randomised, double-blind, placebo-controlled trial of praziquantel treatment during pregnancy. It is also the first trial to examine the effects of praziquantel or of benzimidazole treatment during pregnancy on the broad range of maternal and infant outcomes that might be influenced by pre-natal exposure to helminths. Some of the long-term effects of anthelminthic treatment during pregnancy, including

effects on the offspring's own susceptibility to infection and morbidity when exposed to the same species, have yet to be examined. However, the results to date, taken together with the findings of other recent studies, suggest that the risks and benefits of anthelminthic treatment during pregnancy, and policy regarding routine provision of anthelminthics during antenatal care, may need to be reviewed.

In Entebbe, where good, general antenatal care was provided, there was a possible benefit of albendazole for anaemia in mothers with moderate to heavy hookworm infection, but otherwise none of the expected benefits of anthelminthic treatment on anaemia, birth weight, perinatal mortality, infant

mortality or infant response to immunisation were realised. These findings were in keeping with results from the two other benzimidazole trials (Torlesse and Hodges, 2001; Larocque *et al.* 2006). Mothers with possible symptomatic helminth infection were excluded from our trial, but such women were unusual in our setting: of 11,783 screened for the study only 17 were excluded for haemoglobin below 8 g/dl, 15 for diarrhoea with blood in stools and none for clinically apparent severe liver disease (Webb *et al.* 2011). It other settings, or with other helminth species (for example *S. japonicum*), the balance of benefits to mother and infant may differ. However, the minimal effects associated with helminths and anthelminthic treatment in our setting are put further into perspective by the strong adverse effects of malaria and HIV infection observed; the prevalence of malaria was 13% and of HIV 12% at baseline, during pregnancy, in our study. We found that maternal malaria and HIV infection were strongly associated with maternal anaemia (Muhangi *et al.* 2007), and were also associated with reduced birth weight and increased perinatal mortality (unpublished data). Maternal and infant HIV infection, and infant malaria, were associated with deleterious effects on the infant response to immunisation (Elliott *et al.* 2010).

The three trials have been conducted to date in areas with high hookworm prevalence but low intensity, and this is the common pattern of helminth infection. However, a trial in a setting with heavy hookworm infection might have a different result, especially if background levels of nutrition and iron status are poor. Trial results might also be expected to differ according to helminth species: *Necator americanus* and *Ancylostoma duodenale* may differ in their pathogencity for anaemia (Albonico *et al.* 1998) – and we have not yet determined the prevalent species in Entebbe; schistosome species clearly differ in the immunopathology that they induce (Burke *et al.* 2009). The three reported studies demonstrate that placebo-controlled trials can be conducted safely, and additional trials are still needed in different settings.

The observed adverse effect on infantile eczema is a concern, and contributes to the current equipoise in evidence regarding the potential risks and benefits of anthelminthic therapy during pregnancy. Whether this finding can be generalised to other environments is uncertain: Entebbe is unusual among lake-shore communities in Uganda in being relatively urbanised and traversed by a major highway, and hosting the international airport. Pollutants may contribute to the induction of allergy (Venn *et al.* 2001, 2005), and the results of anthelminthic therapy in such a setting may differ from the results in a purely rural context.

A key question is whether effects on infantile eczema will this translate into an impact on asthma in later life? Studies in affluent countries suggest that while infantile eczema *per se* may not predict later asthma, early development of atopy, and eczema associated with atopy and wheeze in early childhood, may be associated with atopic asthma at school-age (Illi *et al.* 2004, 2006; Williams and Flohr, 2006). Asthma mortality depends not just on prevalence, but also on quality of care; already 80% of asthma deaths occur in low- and middle-income countries (WHO, 2010). It is possible that, as low-income countries develop, anthelminthic treatment programmes will contribute to an epidemic increase in allergic disease similar to that experienced in affluent countries during the 20th century (Bach, 2002). Further research is needed to determine whether this is likely to be the case; if so, measures to equip such countries to manage such an epidemic need to be planned.

Conversely, studies of the underlying mechanisms of helminth-allergy interactions in endemic countries have the potential to provide insights for the development of new tools for the prevention and management of allergic disease, with global health benefits.

REFERENCES

Adam, I., Elwasila, E. and Homeida, M. (2005). Praziquantel for the treatment of schistosomiasis mansoni during pregnancy. *Annals of Tropical Medicine and Parasitology* **99**, 37–40.

Ajanga, A., Lwambo, N. J., Blair, L., Nyandindi, U., Fenwick, A. and Brooker, S. (2006). *Schistosoma mansoni* in pregnancy and associations with anaemia in northwest Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 59–63.

Albonico, M., Stoltzfus, R. J., Savioli, L., Tielsch, J. M., Chwaya, H. M., Ercole, E. and Cancrini, G. (1998). Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* or *Necator americanus*, on iron status of children. *International Journal of Epidemiology* **27**, 530–537.

Allen, H. E., Crompton, D. W., de Silva, N., LoVerde, P. T. and Olds, G. R. (2002). New policies for using anthelmintics in high risk groups. *Trends in Parasitology* **18**, 381–382.

Allen, L. H. (2000). Anemia and iron deficiency: effects on pregnancy outcome. *American Journal of Clinicial Nutrition* **71** (Suppl 5), 1280S–1284S.

Atukorala, T. M., de Silva, L. D., Dechering, W. H., Dassenaeike, T. S. and Perera, R. S. (1994). Evaluation of effectiveness of iron-folate supplementation and anthelminthic therapy against anemia in pregnancy – a study in the plantation sector of Sri Lanka. *American Journal of Clinicial Nutrition* **60**, 286–292.

Bach, J. F. (2002). The effect of infections on susceptibility to autoimmune and allergic diseases. *New England Journal of Medicine* **347**, 911–920.

Brown, M., Mawa, P. A., Joseph, S., Bukusuba, J., Watera, C., Whitworth, J. A., Dunne, D. W. and Elliott, A. M. (2005). Treatment of *Schistosoma mansoni* infection increases helminth-specific type 2 cytokine responses and HIV-1 loads in coinfected Ugandan adults. *Journal of Infectious Diseases* **191**, 1648–1657.

Burke, M. L., Jones, M. K., Gobert, G. N., Li, Y. S., Ellis, M. K. and McManus, D. P. (2009). Immunopathogenesis of human schistosomiasis. *Parasite Immunology* **31**, 163–176.

Christian, P., Khatry, S. K. and West, K. P. Jr. (2004). Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet* **364**, 981–983.

Cooper, P. J., Chico, M. E., Vaca, M. G., Moncayo, A. L., Bland, J. M., Mafla, E., Sanchez, F., Rodrigues, L. C., Strachan, D. P. and Griffin, G. E. (2006). Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. *Lancet* **367**, 1598–1603.

Cruz, M. C. and Edlind, T. (1997). beta-Tubulin genes and the basis for benzimidazole sensitivity of the opportunistic fungus *Cryptococcus neoformans*. *Microbiology* **143**, 2003–2008.

Dayan, A. D. (2003). Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. *Acta Tropica* **86**, 141–159.

de Silva, N. R., Sirisena, J. L., Gunasekera, D. P., Ismail, M. M. and de Silva, H. J. (1999). Effect of mebendazole therapy during pregnancy on birth outcome. *Lancet* **353**, 1145–1149.

Diav-Citrin, O., Shechtman, S., Arnon, J., Lubart, I. and Ornoy, A. (2003). Pregnancy outcome after gestational exposure to mebendazole: a prospective controlled cohort study. *American Journal of Obstetrics and Gynecology* **188**, 282–285.

Doenhoff, M. J., Cioli, D. and Utzinger, J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Current Opinion in Infectious Diseases* **21**, 659–667.

Elliott, A. M., Kizza, M., Quigley, M. A., Ndibazza, J., Nampijja, M., Muhangi, L., Morison, L., Namujju, P. B., Muwanga, M., Kabatereine, N. and Whitworth, J. A. (2007). The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. *Clinical Trials* **4**, 42–57.

Elliott, A. M., Mawa, P. A., Joseph, S., Namujju, P. B., Kizza, M., Nakiyingi, J. S., Watera, C., Dunne, D. W. and Whitworth, J. A. (2003). Associations between helminth infection and CD4+ T cell count, viral load and cytokine responses in HIV-1-infected Ugandan adults. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **97**, 103–108.

Elliott, A. M., Mawa, P. A., Webb, E. L., Nampijja, M., Lyadda, N., Bukusuba, J., Kizza, M., Namujju, P. B., Nabulime, J., Ndibazza, J., Muwanga, M. and Whitworth, J. A. (2010). Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to BCG and tetanus immunisation. *Vaccine* **29**, 247–255.

Elliott, A. M., Mpairwe, H., Quigley, M. A., Nampijja, M., Muhangi, L., Oweka-Onyee, J., Muwanga, M., Ndibazza, J. and Whitworth, J. A. (2005). Helminth infection during pregnancy and development of infantile eczema. *Journal of the American Medical Association* **294**, 2032–2034.

Endara, P., Vaca, M., Chico, M. E., Erazo, S., Oviedo, G., Quinzo, I., Rodriguez, A., Lovato, R., Moncayo, A. L., Barreto, M. L., Rodrigues, L. C. and Cooper, P. J. (2010). Long-term periodic anthelmintic treatments are associated with increased allergen skin reactivity. *Clinical Experimental Allergy* **40**, 1669–1677.

Flohr, C., Quinnell, R. J. and Britton, J. (2009). Do helminth parasites protect against atopy and allergic disease? *Clinical Experimental Allergy* **39**, 20–32.

Flohr, C., Tuyen, L. N., Quinnell, R. J., Lewis, S., Minh, T. T., Campbell, J., Simmons, C., Telford, G., Brown, A., Hien, T. T., Farrar, J., Williams, H., Pritchard, D. I. and Britton, J. (2010). Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. *Clinical Experimental Allergy* **40**, 131–142.

Friedman, J. F., Mital, P., Kanzaria, H. K., Olds, G. R. and Kurtis, J. D. (2007). Schistosomiasis and pregnancy. *Trends in Parasitology* **23**, 159–164.

Haider, B. A., Humayun, Q. and Bhutta, Z. A. (2009). Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. *Cochrane Database of Systematic Reviews* (2), CD005547.

Hall, A. (1981). Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 682–687.

Illi, S., von Mutius, E., Lau, S., Nickel, R., Gruber, C., Niggemann, B. and Wahn, U. (2004). The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *Journal of Allergy and Clinical Immunology* **113**, 925–931.

Illi, S., von Mutius, E., Lau, S., Niggemann, B., Gruber, C. and Wahn, U. (2006). Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* **368**, 763–770.

Keiser, J. and Utzinger, J. (2008). Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *Journal of the American Medical Association* **299**, 1937–1948.

Labeaud, A. D., Malhotra, I., King, M. J., King, C. L. and King, C. H. (2009). Do antenatal parasite infections devalue childhood vaccination? *PLoS Neglected Tropical Diseases* **3**, e442.

Lacey, E. (1990). Mode of action of benzimidazoles. *Parasitology Today* **6**, 112–115.

Lammie, P. J., Hitch, W. L., Walker Allen, E. M., Hightower, W. and Eberhard, M. L. (1991). Maternal filarial infection as risk factor for infection in children. *Lancet* **337**, 1005–1006.

Larocque, R., Casapia, M., Gotuzzo, E. and Gyorkos, T. W. (2005). Relationship between intensity of soil-transmitted helminth infections and anemia during pregnancy. *American Journal of Tropical Medicine and Hygiene* **73**, 783–789.

Larocque, R., Casapia, M., Gotuzzo, E., MacLean, J. D., Soto, J. C., Rahme, E. and Gyorkos, T. W. (2006). A double-blind randomized controlled trial of antenatal mebendazole to reduce low birthweight in a hookworm-endemic area of Peru. *Tropical Medicine and International Health* **11**, 1485–1495.

Leonardi-Bee, J., Pritchard, D. and Britton, J. (2006). Asthma and current intestinal parasite infection: systematic review and meta-analysis. *American Journal of Tropical Medicine and Hygiene* **174**, 514–523.

Lynch, N. R., Hagel, I., Perez, M., Di Prisco, M. C., Lopez, R. and Alvarez, N. (1993). Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *Journal of Allergy and Clinical Immunology* **92**, 404–411.

MacDonald, L. M., Armson, A., Thompson, A. R. and Reynoldson, J. A. (2004). Characterisation of benzimidazole binding with recombinant tubulin from *Giardia duodenalis*, *Encephalitozoon intestinalis*, and *Cryptosporidium parvum*. *Molecular and Biochemical Parasitology* **138**, 89–96.

Malhotra, I., Mungai, P., Wamachi, A., Kioko, J., Ouma, J. H., Kazura, J. W. and King, C. L. (1999). Helminth- and Bacillus Calmette-Guerin-induced immunity in children sensitized in utero to filariasis and schistosomiasis. *Journal of Immunology* **162**, 6843–6848.

Malhotra, I., Mungai, P. L., Wamachi, A. N., Tisch, D., Kioko, J. M., Ouma, J. H., Muchiri, E., Kazura, J. W. and King, C. L. (2006). Prenatal T cell immunity to *Wuchereria bancrofti* and its effect on filarial immunity and infection susceptibility during childhood. *Journal of Infectious Diseases* **193**, 1005–1013.

Malhotra, I., Ouma, J., Wamachi, A., Kioko, J., Mungai, P., Omollo, A., Elson, L., Koech, D., Kazura, J. W. and King, C. L. (1997). *In utero* exposure to helminth and mycobacterial antigens generates cytokine responses similar to that observed in adults. *Journal of Clinical Investigation* **99**, 1759–1766.

Mpairwe, H., Webb, E. L., Muhangi, L., Ndibazza, J., Akishule, D., Nampijja, M., Ngom-Wegi, S., Tumusiime, J., Muwanga, M., Rodrigues, L. C. and Elliott, A. M. (2011). De-worming during pregnancy is associated with an increased risk of allergic conditions in infancy: results from a randomised controlled trial. *Pediatric Allergy and Immunology* **22**, 305–12.

Muhangi, L., Woodburn, P., Omara, M., Omoding, N., Kizito, D., Mpairwe, H., Nabulime, J., Ameke, C., Morison, L. A. and Elliott, A. M. (2007). Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**, 899–907.

Ndibazza, J., Muhangi, L., Akishule, D., Kiggundu, M., Ameke, C., Oweka, J., Kizindo, R., Duong, T., Kleinschmidt, I., Muwanga, M. and Elliott, A. M. (2010). Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. *Clinical Infectious Diseases* **50**, 531–540.

Novato-Silva, E., Gazzinelli, G. and Colley, D. G. (1992). Immune responses during human schistosomiasis mansoni. XVIII. Immunologic status of pregnant women and their neonates. *Scandanavian Journal of Immunology* **35**, 429–437.

Pena-Rosas, J. P. and Viteri, F. E. (2006). Effects of routine oral iron supplementation with or without folic acid for women during pregnancy. *Cochrane Database of Systematic Reviews* **3**, CD004736.

Plotkin, S. A. (2010). Correlates of protection induced by vaccination. *Clinical and Vaccine Immunology* **17**, 1055–1065.

Roche, M. and Layrisse, M. (1966). The nature and causes of "hookworm anemia". *American Journal of Tropical Medicine and Hygiene* **15**, 1029–1102.

Skinner-Adams, T. S., Davis, T. M., Manning, L. S. and Johnston, W. A. (1997). The efficacy of benzimidazole drugs against *Plasmodium falciparum in vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 580–584.

Smits, H. H., Everts, B., Hartgers, F. C. and Yazdanbakhsh, M. (2010). Chronic helminth infections protect against allergic diseases by active regulatory processes. *Current Allergy and Asthma Reports* **10**, 3–12.

Solaymani-Mohammadi, S., Genkinger, J. M., Loffredo, C. A. and Singer, S. M. (2010). A meta-analysis of the effectiveness of albendazole compared with metronidazole as treatments for infections with *Giardia duodenalis*. *PLoS Neglected Tropical Diseases* **4**(5), e682.

Steel, C., Guinea, A., McCarthy, J. S. and Ottesen, E. A. (1994). Long-term effect of prenatal exposure to maternal microfilaraemia on immune responsiveness to filarial parasite antigens. *Lancet* **343**, 890–893.

Torlesse, H. and Hodges, M. (2001). Albendazole therapy and reduced decline in haemoglobin concentration during pregnancy (Sierra Leone). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 195–201.

Tweyongyere, R., Mawa, P. A., Emojong, N. O., Mpairwe, H., Jones, F. M., Duong, T., Dunne, D. W., Vennervald, B. J., Katunguka-Rwakishaya, E. and Elliott, A. M. (2009). Effect of praziquantel treatment of *Schistosoma mansoni* during pregnancy on intensity of infection and antibody responses to schistosome antigens: results of a randomised, placebo-controlled trial. *BMC Infectious Diseases* **9**, 32.

Tweyongyere, R., Mawa, P. A., Ngom-Wegi, S., Ndibazza, J., Duong, T., Vennervald, B. J., Dunne, D. W., Katunguka-Rwakishaya, E. and Elliott, A. M. (2008). Effect of praziquantel treatment during pregnancy on cytokine responses to schistosome antigens: results of a randomized, placebo-controlled trial. *Journal of Infectious Diseases* **198**, 1870–1879.

Utzinger, J., Booth, M., N'Goran, E. K., Muller, I., Tanner, M. and Lengeler, C. (2001). Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology*, **122**, 537–544.

van den Biggelaar, A. H., Rodrigues, L. C., van Ree, R., van der Zee, J. S., Hoeksma-Kruize, Y. C., Souverijn, J. H., Missinou, M. A., Borrmann, S., Kremsner, P. G. and Yazdanbakhsh, M. (2004). Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *Journal of Infectious Diseases* **189**, 892–900.

van Riet, E., Hartgers, F. C. and Yazdanbakhsh, M. (2007). Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology*, **212**, 475–490.

Venn, A., Yemaneberhan, H., Lewis, S., Parry, E. and Britton, J. (2005). Proximity of the home to roads and the risk of wheeze in an Ethiopian population. *Occupational and Environmental Medicine* **62**, 376–380.

Venn, A. J., Yemaneberhan, H., Bekele, Z., Lewis, S. A., Parry, E. and Britton, J. (2001). Increased risk of allergy associated with the use of kerosene fuel in the home. *American Journal of Tropical Medicine and Hygiene* **164**, 1660–1664.

von Mutius, E. and Le Souef, P. N. (2007). Early gene-environment interactions: can they inform primary preventive strategies for asthma? *Seminars in Respiratory Critical Care Medicine* **28**, 255–263.

Webb, E. L., Mawa, P. A., Ndibazza, J., Kizito, D., Namatovu, A., Kyosiimire-Lugemwa, J., Nanteza, B., Nampijja, M., Muhangi, L., Woodburn, P. W., Akurut, H., Mpairwe, H., Akello, M., Lyadda, N., Bukusuba, J., Kihembo, M., Kizza, M., Kizindo, R., Nabulime, J., Ameke, C., Namujju, P. B., Tweyongyere, R., Muwanga, M., Whitworth, J. A. and Elliott, A. M. (2011). Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. *Lancet* **377**, 52–62.

WHO (1995). Report of the WHO informal consultation on hookworm infection and anaemia in girls and women. Geneva, 5–7 December 1994. WHO/CTD/SIP/96.1.

WHO (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.

WHO (2002). Report of the WHO informal consultation on the use of praziquantel during pregnancy/lactation and albendazole/mebendazole in children under 24 months. Geneva, 8–9 April 2002. WHO/CDS/CPE/PVC/2002.4.

WHO (2006). Report of the Scientific Working Group meeting on Schistosomiasis, Geneva, 14–16 November, 2005. TDR/SWG/07.

WHO (2010). *Asthma Facts*. World Health Organisation, Geneva.

Williams, H. and Flohr, C. (2006). How epidemiology has challenged 3 prevailing concepts about atopic dermatitis. *Journal of Allergy and Clinical Immunology* **118**, 209–213.

Woodburn, P. W., Muhangi, L., Hillier, S., Ndibazza, J., Namujju, P. B., Kizza, M., Ameke, C., Omoding, N. E., Booth, M. and Elliott, A. M. (2009). Risk Factors for Helminth, Malaria, and HIV Infection in Pregnancy in Entebbe, Uganda. *PLoS Neglected Tropical Diseases* **3**(6), e473.