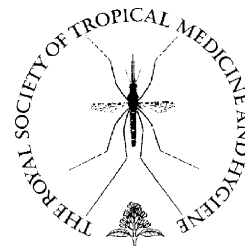




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Ascaris, co-infection and allergy: the importance of analysis based on immunological variables rather than egg excretion

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Summary The ratio of *Ascaris* seropositivity to the presence of eggs in the faeces was 2.44 in children residing near Cape Town, South Africa. Similar and larger ratios have previously been described for children and women living in the city. The new finding strengthens the concept that when helminthic infections occur together with non-helminthic diseases, an analysis of the interaction must include the use of disease-related immunological variables and not be based only on egg excretion status. One of the reasons is that many egg-negative people who live where helminthiasis is highly endemic are immunologically activated as a result of transitory non-patent or intermittent patent infection by *Ascaris* and/or other worms.

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1. Introduction

The question of whether high endemicity of helminthiasis is a factor in the pandemic of HIV/AIDS, as well as having immunomodulatory implications in respect of other infectious and non-infectious diseases, is topical (Adams et al., 2006; Fincham et al., 2003a; Hotez et al., 2006; Obihara et al., 2006). We are concerned that many studies have used and are continuing to use worm egg excretion versus non-

excretion as the only (or main) criterion for attempting to analyse how helminthiasis interacts with diseases such as HIV/AIDS, tuberculosis, malaria, atopic conditions and others (Adams et al., 2006). In communities where numerous people are infected by worms, immunological activation will inevitably be sustained in many egg-negative adolescents and adults by recurrent patent or non-patent (larval) helminthic infection (Adams et al., 2005, 2006). Elevated levels of *Ascaris*-specific IgG are one indication of this kind of activation (Cooper et al., 2004; McSharry et al., 1999; Obihara et al., 2006; Turner et al., 2005). The immunological responses to helminthic antigens have considerable potential to influence other diseases as well as the efficacy of immunisation against non-helminthic infections. Taking

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a substantial body of literature on immunomodulation during helminthiasis into account, it has been argued that the presence or absence of adult worms that produce eggs which are shed in excreta of the host is of uncertain importance for analysis of disease interactions (Adams et al., 2006). This is partly because when *Ascaris* is highly endemic, egg-negative individuals often have strong anti-*Ascaris* T-helper cell type 2 (Th2) immune responses (Turner et al., 2003). On the other hand, in the group in which severe infections occur frequently, Th2 immune responses are usually weak (Jackson et al., 2004) and some individuals are genetically predisposed to infection (Quinnell, 2003).

There are probably also non-immunological ways in which helminths promote the establishment of other infections. For instance, genital schistosomiasis appears to increase the risk of sexually transmitted HIV infection (Kjetland et al., 2006).

2. Results and discussion

It has previously been reported that the seroprevalence of ascariasis in Cape Town, as reflected by *Ascaris*-specific IgE, exceeded egg prevalence by two-fold in women ($n=41$) and three-fold in children ($n=359$) (Adams et al., 2006; Obihara et al., 2006). The people who were studied live in communities where HIV/AIDS is a growing problem. In the women, the presence of eggs in faeces (as determined by examination of several faecal samples from each person) and *Ascaris*-specific IgE were independent variables (Adams et al., 2006). However, the presence of eggs in faeces of the children (based on two faecal samples per child) was positively associated with raised *Ascaris*-specific IgE (adjusted odds ratio 2.4, 95% CI 1.3–4.5) (Obihara et al., 2006).

We now have data from an additional group of 52 randomly selected children (screened for all gastrointestinal helminths) living in a rural environment not far from Cape Town. In all of these groups, *Ascaris*-specific IgE was measured by the same fluorescent enzyme immunoassay method (FEIA CAP RAST; Pharmacia®, Uppsala, Sweden). The assay uses whole-worm *Ascaris suum* antigen, which is suitable because the protein profile of *A. suum* is almost identical to that of *A. lumbricoides* (Abebe et al., 2002). Twenty-two of the rural children were seropositive in terms of raised *Ascaris*-specific IgE and nine had *Ascaris* eggs in their faeces, a ratio of 2.44. Kendall's rank correlation coefficient for egg count/g of faeces and *Ascaris*-specific IgE was $\tau=0.481$ ($P<0.01$). Rank correlation was necessary because there were many ties (equal values) in the variables. This result supports the positive association found in the urban children (Obihara et al., 2006). In the rural children, egg counts and *Ascaris*-specific IgE were not influenced by age (range 7–14 years) or gender. *Ascaris* egg prevalences and 95% CIs in the groups were: rural children 17.3% (9/52), 95% CI 9.4–29.7; urban children 14.8% (53/359), 95% CI 11.5–18.8; and urban women 24.4% (10/41), 95% CI 13.8–39.3.

The results from the new group of rural children and those from the urban women and children were all for communities living under varying degrees of socioeconomic deprivation in or close to Cape Town. Taken together, the data confirm that diagnosis of ascariasis by detection of egg excretion at any given time only partly reflects ongoing

immunological activation caused by *Ascaris* in the communities concerned. It follows that the position will be similar for other worms, including schistosomes, elsewhere in South Africa (and in many other countries) where the endemicity of helminthiasis is even greater than that which has recently been described for Cape Town (Adams et al., 2005). However, in some densely populated, underserved parts of the city where people live in shacks, the prevalence of egg-positive ascariasis in children attending primary schools has been found to be as high as 75%, usually with *Trichuris* co-infection (Adams et al., 2005).

In Cameroon, India, Nigeria and Tanzania, where the burden of *Ascaris* worms carried by children and the frequency of infection generally exceed those reported from Cape Town, studies have shown the importance of measuring *Ascaris*-specific IgE and IgG4 in egg-negative groups, as well as cytokines (Jackson et al., 2006; Malla et al., 2006; McSharry et al., 1999; Turner et al., 2005). In South Africa, regular deworming has reduced eosinophilia, which is also a marker for immunological activation due to infection by worms (Fincham et al., 2003b). Research in Ecuador revealed that 98.5% of a group of 132 school-age children had detectable levels of *Ascaris*-specific IgG1, which was interpreted as reflecting universal exposure to *Ascaris* in the population, including egg-negative individuals (Cooper et al., 2004). Amongst 2865 children living in the same area, significant interaction between *Ascaris*-specific IgG4 and a positive skin test considered to be diagnostic for atopy was independent of egg excretion (Cooper et al., 2003). To what extent cross-reaction between *Ascaris* antigens and various allergens (helminthic and non-helminthic) may lead to false-positive serological results is not yet clear (Arruda and Santos, 2005; Cooper et al., 2004). *Trichuris* occurs in Cape Town and hookworm is rare (Adams et al., 2005), but schistosomiasis and filariasis are not endemic.

Despite the fact that many egg-negative people will be immunologically worm-positive in areas where helminthiasis is highly endemic, researchers have often attempted to analyse possible interactions during co-infection by worms and other pathogens, as well as with atopic conditions, by comparison of variables measured in groups of people selected on the basis of egg excretion versus non-excretion. Postulations that egg positivity may confer some degree of susceptibility or protection in respect of bystander infections or conditions frequently ignore the fact that the absence of eggs may indicate the presence of strong Th2-polarised, antihelminth immune responses that are likely to influence co-infections, allergy and the efficacy of immunisation (Adams et al., 2006; Cooper et al., 2003; Fincham et al., 2003a, 2003b). To reach valid conclusions, ongoing and future analyses must incorporate disease-related immunological variables, including those that specifically reflect responses to helminthic antigens. It must be recognised that elucidation of potential interactions that are influenced strongly by immune responses and genes cannot be based mainly on egg excretion status.

Although studies to define helminth-associated immunological differences between categories (e.g. seropositive, egg-negative and egg-positive) and contexts of interest (e.g. high risk of HIV infection) will be relatively costly in terms of the technology needed to measure immunological variables in sequential blood samples, it should be

possible to economise by minimising the number of people in controlled, replicated experiments. Several research approaches will be useful. Small groups of children and adults known to be susceptible and resistant, respectively, to helminthic infection could be studied during regular deworming both in egg-positive and egg-negative phases. Taking age, gender and intensity of infection into account, analyses will need to establish whether variables such as cytokine levels, specific Igs and eosinophilia are changed by set treatment regimens and in relation to the different types of worm infection.

However, the need for large-scale faecal surveys to determine the prevalence of egg-positive helminthiasis will remain for various reasons. World Health Assembly (WHA) members have agreed to implement control of schistosomiasis and soil-transmitted helminths by regular mass therapy and other methods as an affordable intervention to improve child and public health as well as education (Fincham et al., 2005); and helminthiasis is included in the neglected tropical diseases initiative (Hotez et al., 2006). In terms of the WHA recommendations, the best way to check the effectiveness of service delivery to the public is to run sentinel surveys of faecal samples collected at sites where infection prevalence has historically been high. Development of resistance to anthelmintics is a serious emerging problem, which makes it essential to monitor the efficacy of treatment. Moreover, clinical trials to test new anthelmintics will continue to be necessary (Fincham et al., 2005). Despite the parasitological importance of egg prevalence data, they are of limited value for analysis of immunological interactions between helminthiasis and bystander diseases.

Authors' contributions: JEF conceived and designed the study; MBM searched the literature; LVDM performed the statistical analysis; MEVS and VJA obtained ethical permission for the school-based survey and conducted the fieldwork and liaison; VJA carried out microscopy of faecal samples; MAD is a paediatrician and was responsible for ethical monitoring; JEF interpreted the data; JEF and MBM wrote the paper and responded to the reviewers. All authors read and approved the final manuscript. JEF and MBM are guarantors of the paper.

Conflicts of interest statement: The authors have no conflicts of interest concerning the work reported in this paper.

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