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**Diagnosis, transmission and immunology of human
Oesophagostomum bifurcum and hookworm infections in
Togo**

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Citation

Pit, D. S. S. (2000, October 12). *Diagnosis, transmission and immunology of human Oesophagostomum bifurcum and hookworm infections in Togo*. Retrieved from <https://hdl.handle.net/1887/13934>

Version: Corrected Publisher's Version

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Chapter 4

Antigen specific IgG4 and IgE responses in individuals infected with *Oesophagostomum bifurcum* and *Necator americanus*

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SUMMARY

Possible infections with *O. bifurcum* may not be recognised in other regions of the world, because the eggs of *O. bifurcum* and hookworm are morphologically identical. Diagnosis is mostly based on coproculture but that method has a number of serious shortcomings. First, it can be performed on fresh stool samples only and is quite fastidious and requires some practice to differentiate the larvae. Secondly, due to considerable intra-specimen and day-to-day variations in the egg output, light infections can easily be missed. Thirdly, pathology of *O. bifurcum* infections is not caused by the lumen-dwelling worm but by the immunogenic larvae encapsulated in the intestinal wall.

Diagnosis based on the detection of parasite specific antibodies would enable us to screen populations where hookworm-like eggs are commonly found in the stools. In this study, sera were collected in *O. bifurcum* endemic villages in northern Togo, and in non-endemic villages in central Togo. *O. bifurcum* specific IgG₄ antibodies were found in sera from northern Togo as well as from central Togo. When the serum was preabsorbed with *N. americanus* coated beads, the *O. bifurcum* specific antibodies were absent in the sera from central Togo, suggesting that there is cross-reactivity between *N. americanus*-specific IgG₄-antibodies and *O. bifurcum* antigen. In contrast, *O. bifurcum* specific IgE antibodies were detected in sera from individuals from northern Togo, but not in central Togo.

INTRODUCTION

In northern Togo, infections with hookworm (*Necator americanus*) and *Oesophagostomum bifurcum* are highly endemic (Polderman *et al*, 1991, Pit *et al*, 1999b). The exact life cycle of *O. bifurcum* is unknown, but accumulating evidence suggest that after oral ingestion, the L3 larvae encapsulate in the intestinal wall, where they might go into arrested larval development (ALD) for a more or less prolonged period of time, before re-entering the intestinal

lumen. In the lumen the larvae rapidly mature to young adult worms, mate and start egg production (Polderman *et al*. 1991). In some patients later larval stages, i.e. immature juvenile worms, can be found in the bowel wall. It is not sure whether these larvae failed to return to the lumen or reinvaded the intestinal wall at a later stage. These juveniles induce a highly immunological reaction and cause pathology known as "Tumeur de Dapaong".

Diagnosis based on detection of *O. bifurcum* and hookworm eggs is not parasite specific, therefore a copro-culture has been developed, such that the infective larvae of both nematodes can be differentiated through their morphological features (Blotkamp *et al.*, 1993). This method, which only detects infections with adult lumen-dwelling worms, is quite fastidious and requires some practise to differentiate the larvae. As a result possible infections with *O. bifurcum* might not be recognised in other hookworm-endemic regions of the world. In addition, due to considerable intra-specimen and day to day variations in the egg output, light infections can easily be missed (Pit *et al.*, 1999a), and fresh stool samples are required. Infections with tissue-dwelling larval stages only, as frequently seen in clinical cases, are also missed in coproculture. Diagnosis based on the detection of parasite specific antibodies would enable us to screen populations where hookworm like eggs are commonly found in the stools, and to detect clinical cases where the larvae are still encapsulated in the intestinal wall.

A recent study has shown that chronic infections with *O. bifurcum* as well as with hookworm induce a Th2-type immune response (Pit *et*

al., manuscript in preparation), generally associated with raised levels of IgE and IgG₄ (Mosmann & Coffman, 1989).

A IgG₄-specific enzyme linked immunosorbent assay (ELISA) was developed to diagnose human infections with *O. bifurcum* (Polderman *et al.*, 1993). Observations on small numbers of samples suggested that specificity and sensitivity were quite satisfactory but, the precise sensitivity could not be determined and possible cross-reactivity between *O. bifurcum* and *N. americanus* antibodies could not be excluded.

The aim of the present study was to evaluate the advantage of the IgG₄-specific ELISA as a diagnostic tool to determine the prevalence of infection with *O. bifurcum* in two different populations in Togo: one in which both *O. bifurcum* and hookworm are transmitted and another one with endemic hookworm infections only.

The applied immunodiagnosis was less specific than expected, and there was cross-reactivity between *N. americanus* specific IgG₄ and *O. bifurcum* antigen. Pritchard and Walsh (1995) reported that IgE antibodies showed minimal cross-reactivity against antigens from different nematode species. Similarly, there

was significantly less cross-reactivity with heterologous parasite antigens in the IgE antibody response to filarial infection than in the corresponding IgG antibody response (Weiss *et al*, 1982). We therefore studied the possibility to measure IgE antibodies against *O. bifurcum* in sera from individuals in Togo as a specific diagnostic tool.

MATERIAL AND METHODS:

Study population and parasitological examination.

Serum and stools were obtained from volunteers coming from *O. bifurcum*

and *N. americanus* endemic villages around Dapaong and from volunteers from villages from Central Togo, where *N. americanus* is highly endemic but where *O. bifurcum* infections have never been found despite extensive parasitological surveys. Informed consent was obtained from the patients or their parents. The parasitological and demographic characterisation of the population is given in table 1.

Table 1: Demographic and parasitological characterisation of the study population.

	Village	km from Dapaong	n=	male/ female	Median-age (range)	% infected with	
						<i>O. bifurcum</i>	<i>N. americanus</i>
1	Gossiété	22	40	21/20	24 (7-65)	80	80
2	Sintodi	25	45	21/24	26 (5-69)	27	80
3	Tonte	14	50	27/23	28 (6-61)	48	80
4	Ogaro	35	38	21/17	24 (5-70)	46	86
5	Lotogou	27	23	9/14	23 (11-57)	26	87
6	Galé	21	30	24/6	38 (20-70)	0	70
7	Mogou	75	34	21/13	32 (10-55)	0	68
8	Sokodé	280	38	1/37	28 (17-43)	0	45
9	Sagbadai	300	46	32/14	39 (11-71)	0	48
10	Dutch donors		30		Unknown	0	0

Sera from Dutch anonymous donors were used as a reference. Infections with *O. bifurcum* and *N. americanus* were detected by stool cultures as previously described (Polderman *et al.*, 1991). Briefly, three grams of stools from all individuals (except the anonymous Dutch donors) were cultured in the moist environment of a petri-dish. After seven days egg-hatched larvae were collected, identified as *O. bifurcum* or *N. americanus* and counted.

Preparation of *O. bifurcum* (Oesag) and *N. americanus* (Necag) antigens

Following treatment of patients with pyrantel pamoate and purgation, adult worms of *O. bifurcum* and *N. americanus* were isolated as described by Polderman *et al.* (1991). Isolated adult worms were extensively washed in PBS (Phosphate-buffered saline, pH 7.6), lyophilised, ground with a mortar and pestle and then extracted in 0.035 M PBS overnight with gentle stirring at 4°C. The protein concentration was determined with a BCA protein assay (Pierce).

Serological assays.

O. bifurcum- or *N. americanus*-specific IgG₄ in patients' sera was

determined as described by Polderman *et al.* (1993).

For the determination of *O. bifurcum*- or *N. americanus*-specific IgE in patients, sera were preabsorbed with protein G (Pharmacia, Uppsala, Sweden) to eliminate IgG antibody competition, as previously described by Quinnell *et al.* (1995). Briefly, diluted sera (1:4) in 0.035M PBS (pH 7.8) were incubated on a rotor with an equal volume of Protein G at 4°C overnight. Thereafter, samples were centrifuged for 15 min and supernatants collected. Microtitration plates (Maxisorb, Nunc) were coated with *O. bifurcum*- and *N. americanus*-specific antigen at 5 µg protein/ml in 0.1M sodium carbonate buffer (pH 9.6) overnight at 4°C (*Oesag* and *Necag* respectively). Plates were blocked with PBS containing 1% bovine serum albumin for 1 h at 37°C, then washed with 0.035M PBS and preabsorbed serum (final dilution 1:40) was added in duplicates and incubated at 4°C overnight. After washing (as above), anti-human IgE mouse monoclonal antibody (Sigma) was used, followed by AP-conjugated rabbit anti-mouse antibody (Sigma) (1 h at 37°C), and after incubation with p-nitrophenyl phosphate (pNPP) for 1 h at room

temperature absorbance was read at 405 nm. To correct for assay variation, results were expressed as ratios between the absorbance values of samples and defined control sera.

Preabsorption of the sera with *N. americanus* antigen (Necag)

Beads in sepharose gel were activated with Cyanogenbromide (CnBr) after having been washed with bidest on a glass filter. After a second washing the beads were incubated with *N. americanus*-antigen (Necag, 1mg/ml) at room temperature overnight with gentle rocking. The coupled beads were then washed with PBS and deactivated overnight at room temperature in a solution of 0.5M 4-aminobutyricacid ($C_4H_9NO_2$) and 0.05 M Na_2CO_3 (pH 10). After being washed in PBS, the coupled beads were stored in the refrigerator until use.

For the absorption of *N. americanus* specific antibodies in the serum, the coupled beads were washed twice and incubated overnight at 4°C with 1/40 diluted serum, while rocking. The next morning the serum and coupled beads mixture was centrifuged and the supernatant (= pre-absorbed serum) was used in an *O. bifurcum*-specific-IgG4-ELISA as

described before (Polderman *et al*, 1993).

RESULTS:

IgG4 antibodies against *O. bifurcum* and *N. americanus*.

O. bifurcum specific IgG₄ levels were determined in patients from six villages in the *O. bifurcum* endemic area in northern Togo and in control patients from three villages in the non-endemic region of central Togo. Subjects from the *Oesophagostomum*-endemic area showed high levels of IgG4 against *O. bifurcum* antigen in their serum. The prevalence of infections measured in northern Togo with ELISA was higher than measure with the stool cultures (respectively 73% and 40% positive). In Galé, a village where all coprocultures remained negative for *Oesophagostomum*, 14 out of 30 subjects tested were IgG4-positive. Low levels of reactivity were seen in two of the non-endemic villages but not in the third one, where 18 out of 44 were IgG4 positive. (figure 1a).

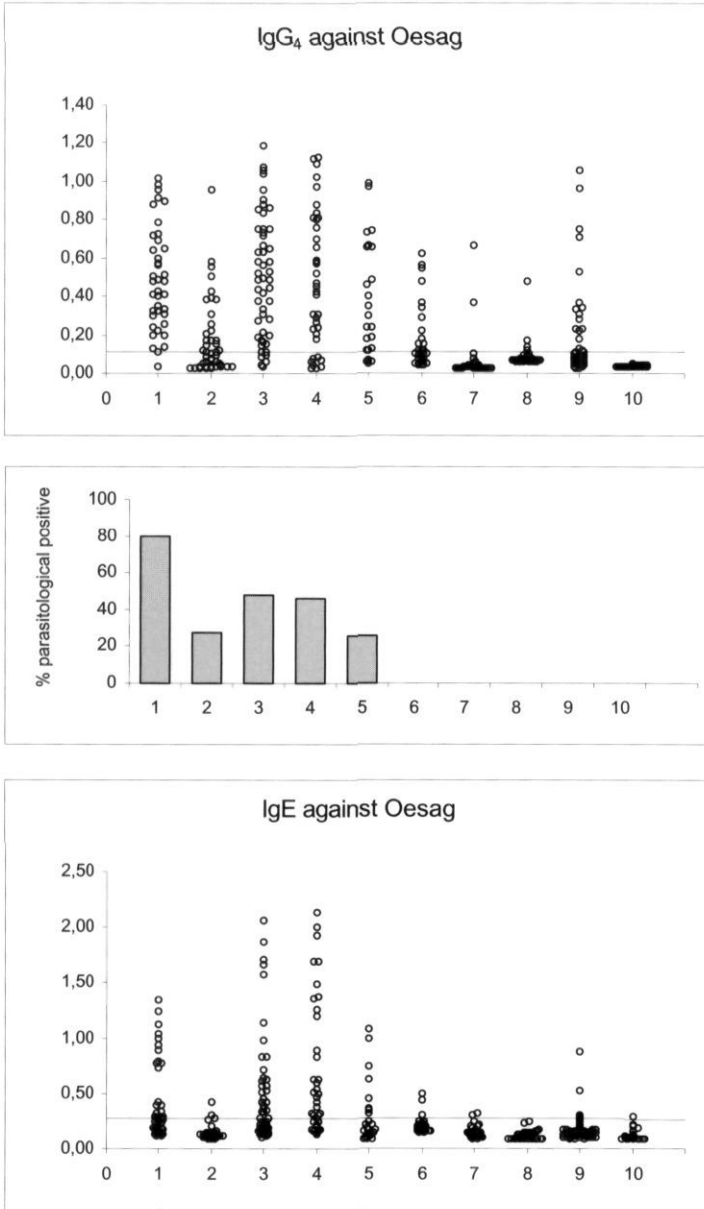


Fig. 1: Absorbance values in ELISA specific for IgG₄ (a) and IgE (b) against *O. bifurcum* antigen in serum from endemic and non-endemic patients. The prevalence of infection with *O. bifurcum* of the patients-group per village is given in the central graph. The characteristics of the 10 patients groups are given in Table 1

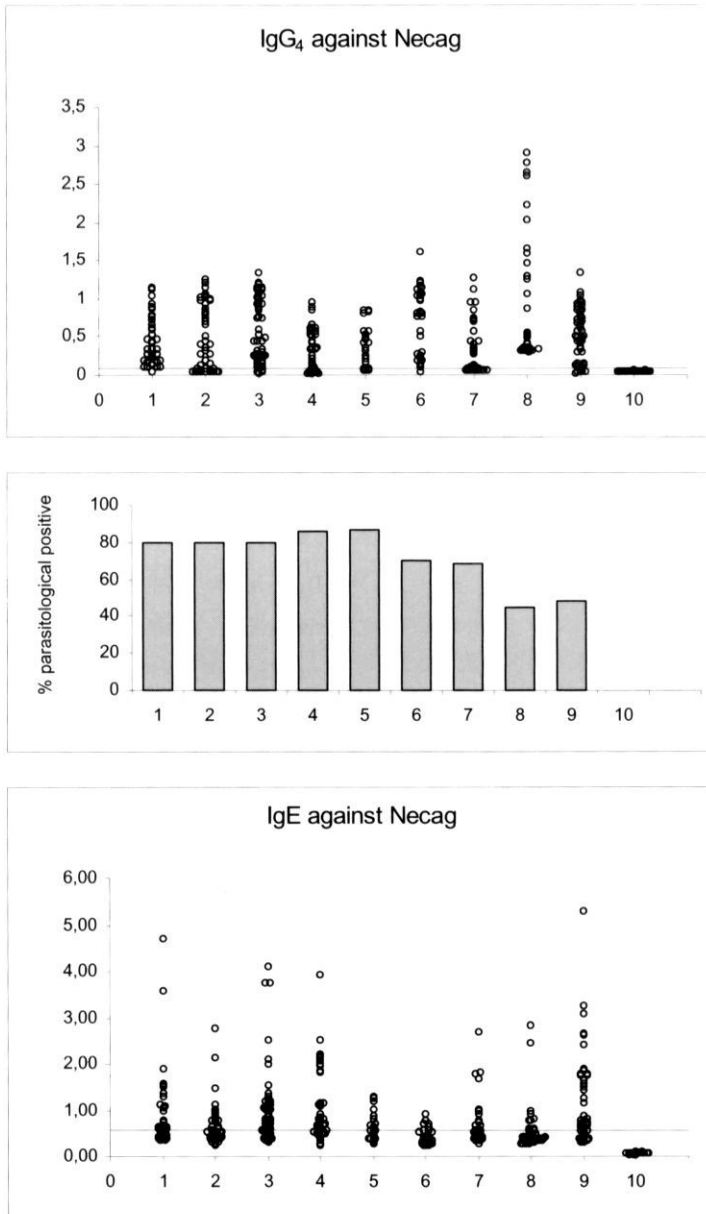


Fig. 2: Absorbance values in ELISA specific for IgG₄ (a) and IgE (b) against *N. americanus* antigen in serum from *O. bifurcum* endemic and non-endemic patients. The prevalence of infection with *N. americanus* of the patients-group per village is given in the central graph. The characteristics of the 10 patients groups are given in Table 1

In total 20% of the patients from central Togo showed IgG4 reactivity against *O. bifurcum* antigen. Subjects of both populations had high levels of specific anti-hookworm IgG4 (figure 2a), and prevalence of infection measured by ELISA (84% positive) was higher than by stool culture (71% positive). No reactivity was seen in the Dutch controls, neither for *Oesophagostomum* nor for hookworm.

Preabsorption of the serum with *N. americanus* antigen.

Preabsorption of the sera with *N. americanus* antigen showed that *N. americanus* specific antibodies were binding *Oesophagostomum* antigen (figure 3). Indeed, the levels of *O. bifurcum* specific IgG₄ antibodies remained high in the sera from patients from northern Togo, after absorption with *N. americanus* antigen. *O. bifurcum* specific IgG₄ levels from patients from central Togo, on the other hand, decreased after absorption of the sera with *N. americanus* antigen.

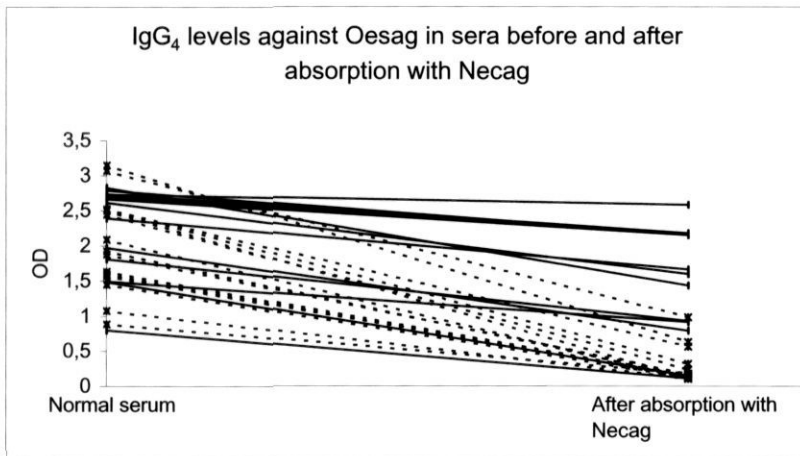


Fig. 3: Absorbance values in ELISA specific for IgG₄ against *O. bifurcum* in normal serum and in serum pre-absorbed with *N. americanus* antigen. The straight line corresponds to values found in sera from individuals from northern Togo, the dotted line to serum from individuals from central Togo.

IgE antibodies against *O. bifurcum* and *N. americanus* antigen

O. bifurcum specific IgE antibodies were detected in the serum from individuals from northern Togo, but much less in central Togo (figure 1b). Prevalence of infections measured in northern Togo by ELISA (37%) was similar to the prevalence found by coproculture (40%). In central Togo IgE antibodies against *O. bifurcum* were detected in only 6% of the patients. *N. americanus* specific IgE antibodies were detected in serum of patients from both northern and central Togo (figure 2b). But on a population level prevalence of infection measured by ELISA was lower than when measured by coproculture (respectively 45% and 53% positive).

DISCUSSION

IgG4 is known to be a marker of (chronic) antigen exposure, in particular to chronic helminth infections (Aalberse *et al.*, 1985). In a previous study, Polderman *et al.* (1993) described an ELISA to diagnose IgG4 antibodies against *O. bifurcum* in humans. Small scale testing suggested the procedure to be fairly specific and sensitive but more extensive experience was required. Therefore it has been our first choice anti-

body for the development of a diagnostic method based on a parasite-specific ELISA. Our current results, however, show that some patients from a neighbouring, non-endemic region also had high serum levels *O. bifurcum*-specific IgG4 antibodies. These serological results are inconsistent with our parasitological results from those patients.

The lack of specificity could be explained in different ways. First, it is likely that a nematode ill adapted to the human host, like *Oesophagostomum* species may be able to invade patients without reaching adulthood. Coproculture remains negative but serology may become positive. The high rates of false positive findings in Gale, in the oesophagostomum endemic region, should perhaps be explained in this way but the high rates of specific IgG4-positivity in one village in central Togo is difficult to explain.

Alternatively, cross-reactivity between antigens of different parasite species may occur, in the test system used. Indeed, such cross reactivity has been demonstrated for many parasites (Weiss *et al.*, 1982; Correa-Olivera *et al.*, 1988). Our absorption studies on the IgG4 antibodies also showed cross-reactivity between *N. americanus* specific IgG4 and *O. bi-*

furcum antigen. Of course, all sera could be preabsorbed with *N. americanus*-coated beads before performing an *O. bifurcum*-specific ELISA, but this is time as well as antigen consuming, thus not applicable on a large scale. Sera could not be preabsorbed with *N. americanus* directly (data not shown). Therefore IgG₄ can not be used as a specific antibody for the diagnosis of *O. bifurcum* infections in a hookworm endemic area.

Greater specificity of IgE for helminth infections has been reported previously (Pritchard & Walsh, 1995; Ganguly *et al.*, 1988; Weiss *et al.*, 1982). Our results also indicate a lesser degree of cross-reactivity between *N. americanus* specific IgE antibodies and *O. bifurcum* antigen. Unfortunately sensitivity and specificity could not be calculated because the parasitological diagnosis is not sufficiently reliable. However, our data suggest that the IgE specific ELISA is, although more specific, not very sensitive. On an individual level parasitological and IgE levels do not correspond, but on a village level the prevalence of infection measured with both diagnostic tools are comparable. It is still remarkable and unexplained why there was no cross-reactivity in other regions (Ghana, Zaire) measured previously

(Polderman *et al.*, 1993), when there obviously was cross-reactivity in this area.

It was not possible to evaluate the value of measuring levels of IgG₄ and IgE against *N. americanus*, because hookworm is endemic over the whole region and it's not possible to find endemic controls.

In conclusion, the use of an IgG₄ specific ELISA in hookworm endemic area's for the diagnosis of *O. bifurcum* is hampered by the cross-reactivity between *N. americanus* specific IgG₄ and *O. bifurcum* antigen. The IgE specific ELISA against *O. bifurcum* is more specific but should be used on a village level rather than on an individual level.

Acknowledgements

We would like to thank Mrs Assibi Lamboni & Mr Etienne Yark for their assistance in the field, and Nathalie Cani & Blandine Bizieux for their help with the blood collection. The study was funded by the Netherlands Foundation for Advancement of Tropical Research (WOTRO).

REFERENCES:

Aalberse R.C., van der Gaag R., van Leeuwen J: Serologic aspects of IgG₄ antibodies. I. prolonged im-

- munization results in an IgG4-restricted response. *J. immunology*; 130-722.
- Blotkamp J, Krepel HP, Kumar V, Baeta S, van't Noordende JM, Polderman AM. (1993). Observations on the morphology of adults and larval stages of *Oesophagostomum* sp isolated from man in northern Togo and Ghana. *Journal of Helminthology*;67:49-61.
- Correa-Oliveira R., Dusse L.M.S., Viana I.R.C., Colley D.G., Carvalho O.S., and Gazzinelli G. (1988). Human antibody responses against schistosomal antigens. *Am. J. Trop. med hyg.* 38(2) 348-355
- Ganguly N.M., Mahajan R.C., Sehgal R., Shetty P., & Diawari J.B. (1988). Role of specific immunoglobulin E to excretory-secretory antigen in diagnosis and prognosis of hookworm infection. *J. of Clinical Microbiology*, 26, 737-742.
- Mosmann T.R., and Coffman R.L. (1989). Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol.* 7: 145-73
- Pit D.S.S., de Graaf W., Snoek H., de Vlas S.J., Baeta S.M., and Polderman A.M. (1999a). Diagnosis of *Oesophagostomum bifurcum* and hookworm infection in humans: day-to-day and within-specimen variation of larval counts. *Parasitology*, 118, 283-288.
- Pit D.S.S., Rijckers F.E.M, Rasport E.C., Baeta S., & Polderman A.M. (1999b). Geographical distribution of *Oesophagostomum bifurcum* and hookworm infections in northern Togo. *American Journal of Tropical Medicine and Hygiene*, 61(6), p 951-955.
- Polderman AM, Krepel HP, Baeta S, Blotkamp J & Gigase P. (1991). Oesophagostomiasis, a common infection of man in northern Togo and Ghana. *American Journal of Tropical Medicine and Hygiene*;44(3):336-44
- Polderman AM, Krepel HP, Verweij JJ, Baeta S, Rotmans JP (1993). Serological diagnosis of *Oesophagostomum* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 87:433-435
- Pritchard D.I., Walsh E.A. (1995). The specificity of the human IgE response to *Necator americanus*. *Parasite immunology*, 17, 605-607.
- Quinnell R.J., Woolhouse M.E.J., Walsh E.A., Pritchard D.I. (1995). Immunoepidemiology of human

necatoriasis: correlation between antibody responses and parasite burdens. *Parasite immunology*; *17*, 313-318

Weiss N., Hussain R., Ottesen E.A. (1982). IgE antibodies are more species specific than IgG antibodies in human onchocerciasis and lymphatic filariasis. *Immunology*, *45*, 129-137