Antigenic variability in *Trichuris trichiura* populations

R. M. CURRIE[1]*, C. S. NEEDHAM[1], L. J. DRAKE[1], E. S. COOPER[2] *and* D. A. P. BUNDY[1]

[1] *The Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX*1 *3PS, UK*
[2] *Tropical Metabolism Research Unit (TRMU), University of the West Indies, Kingston* 7, *Jamaica*

(*Received 18 February 1998; revised 14 April 1998; accepted 14 April 1998*)

SUMMARY

The present study examines antigenic variability for the human whipworm *Trichuris trichiura*. Recognition by IgG of somatic antigens of individual worms collected from 3 intensely infected children from Jamaica, West Indies has been investigated by immunoblotting. When probed with 1 plasma sample, significant differences in recognition of 2 selected antigens among worm populations and between male and female worms was observed. In addition, there was evidence for antigenic variability within worm populations at the individual worm level. Such variation may have considerable implications for the development of immunity to parasitic nematodes.

Key words: *Trichuris trichiura*, SDS–PAGE, immunoblotting, antigenic variability.

INTRODUCTION

The existence of considerable host heterogeneity in immunological response to helminth infection is widely recognized (Haswell-Elkins *et al*. 1989, 1992; Kennedy *et al*. 1990; Needham *et al*. 1993). However, the degree to which the expression of antigens varies within a parasite population of a given species of parasite is an area of helminth immunobiology which is poorly understood. The present study examines antigenic variability within a population of human whipworm (*Trichuris trichiura*) by comparing antigen recognition by IgG of individual *T. trichiura* worms collected from 3 heavily infected children from Jamaica, West Indies.

MATERIALS AND METHODS

Field component

Adult *T. trichiura* were obtained by oxantel (Pfizer) treatment of *Trichuris* dysentery syndrome (TDS)-hospitalized children as part of an ongoing clinical trial in Jamaica, approved by the ethical committee of the Faculty of Medicine, University of the West Indies. Expelled worms were washed, counted and frozen separately for each individual. All worms were kept at −70 °C for long-term storage. A peripheral blood sample was also collected from each subject with the plasma fraction separated and stored at −20 °C. Adult *T. trichiura* worms used in this study were collected from 3 TDS-hospitalized children (worm populations 1, 2 and 3); plasma samples used for immunological analyses were from 2 different TDS-hospitalized children (designated A and B).

* Corresponding author: The Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK. Tel: +01865 281229. Fax: +01865 281245. E-mail: rachel.currie@zoology.oxford.ac.uk

Preparation of individual adult T. trichiura *worm extract*

T. trichiura adult worm extracts were prepared from individual worms collected from 3 children (populations 1, 2 and 3). Worms were weighed and ground in SDS–PAGE reducing sample buffer (15% glycerol, 3% SDS, 0·125 M Tris, pH 6·8, 5% β-mercaptoethanol) in individual tissue homogenizers (Scotlab, UK; 1·5 ml volume capacity), at a standard concentration of 1 mg wet weight *T. trichiura* worms/50 μl of sample buffer. Individual worms varied in weight, therefore the volume of sample buffer added was dependent on the weight of the individual worm. Each individual worm was ground for exactly 3 min to standardize the extraction process. Following centrifugation of samples at 12000 ***g*** for 30 min at 4 °C, the supernatants were removed and taken as individual worm extracts.

SDS–PAGE and immunoblotting

Immunoblotting was used to examine antigen recognition of individual worm extracts by IgG antibodies in plasma from 2 children. Individual worm extracts were separated on 12·5% polyacrylamide gels using the BRL large gel system. On each gel, protein extracts of 8 individual male and 8 individual female worms collected from the same child were electrophoresed. To allow comparison between in-

 © 1998 Cambridge University Press
https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press

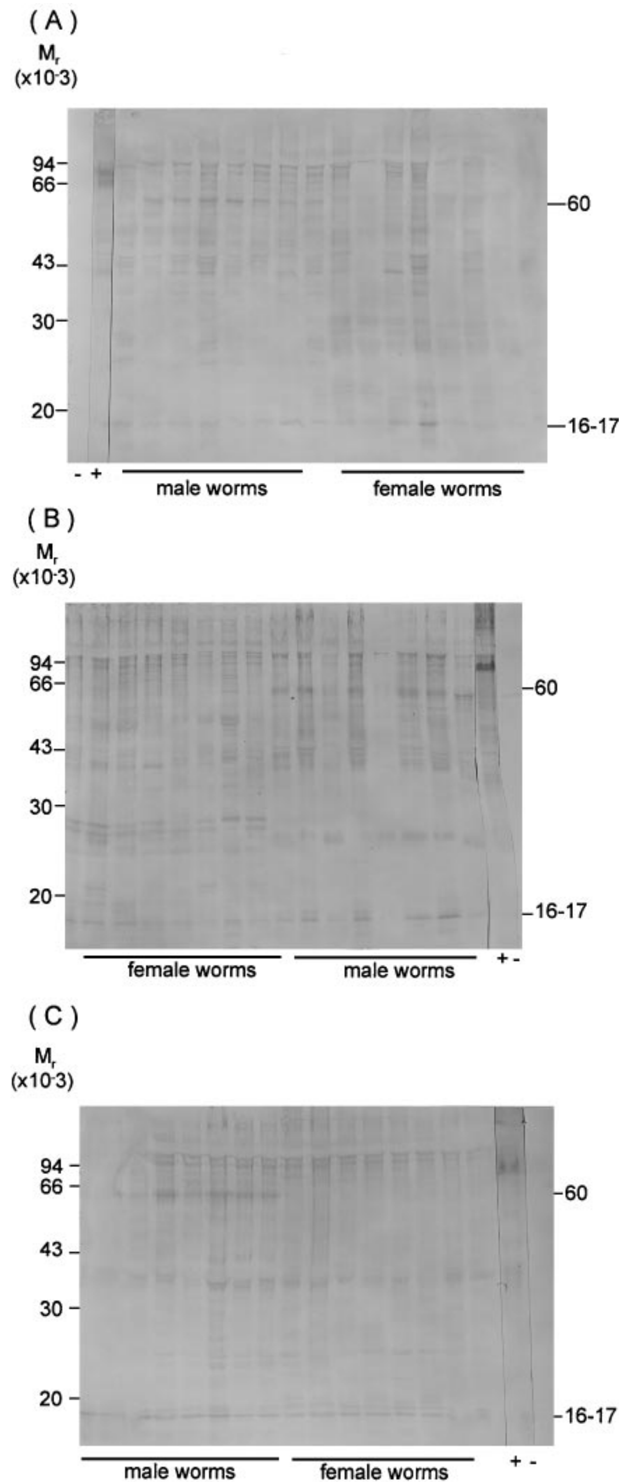

Fig. 1. Antigen recognition profiles of the 3 *Trichuris trichiura* populations by IgG antibodies of plasma A. Each lane represents recognition of antigens from an individual worm with 8 male worms and 8 female worms each from the same population included for each blot (A–C). Responses selected for quantification are indicated (molecular weights are expressed as kDa). Positive (+) and negative (−) controls are shown on each blot.

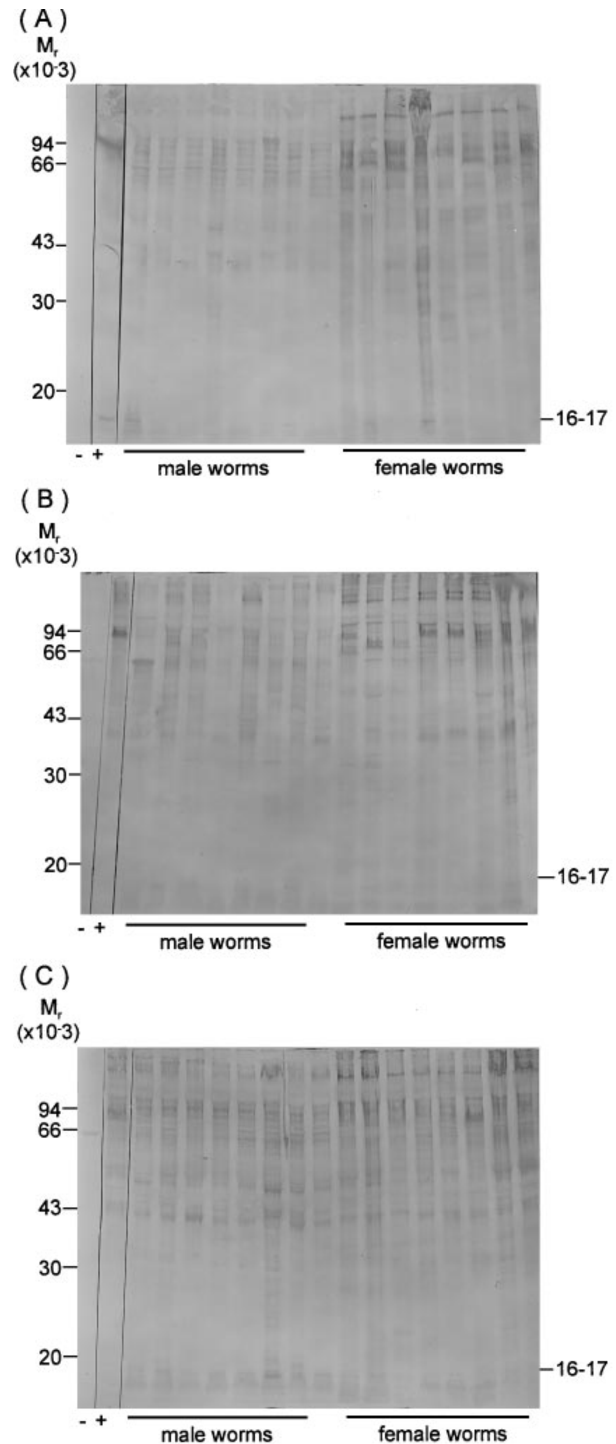

Fig. 2. Antigen recognition profiles of the *Trichuris trichiura* populations by IgG antibodies of plasma B. For other details, refer to the legend for Fig. 1.

dividual worms, 20 μl of individual worm extracts was loaded. Following electrophoresis the separated components were transferred to nitrocellulose paper (NCP; Schleicher & Schuell, BA85) using a discontinuous buffer system and semi-dry blotting apparatus for 2 h at 194 mA. Proteins were visualized using Ponceau S solution (Sigma, UK), destained in water for precisely 15 sec and photographed. The NCP was blocked overnight at 4 °C in 10% foetal calf serum (FCS) in Tris saline/Tween (TBS; 10 mM Tris, 14 mM NaCl, 0·05% Tween 20, pH 8·5), then incubated for 2 h with serum diluted

https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press

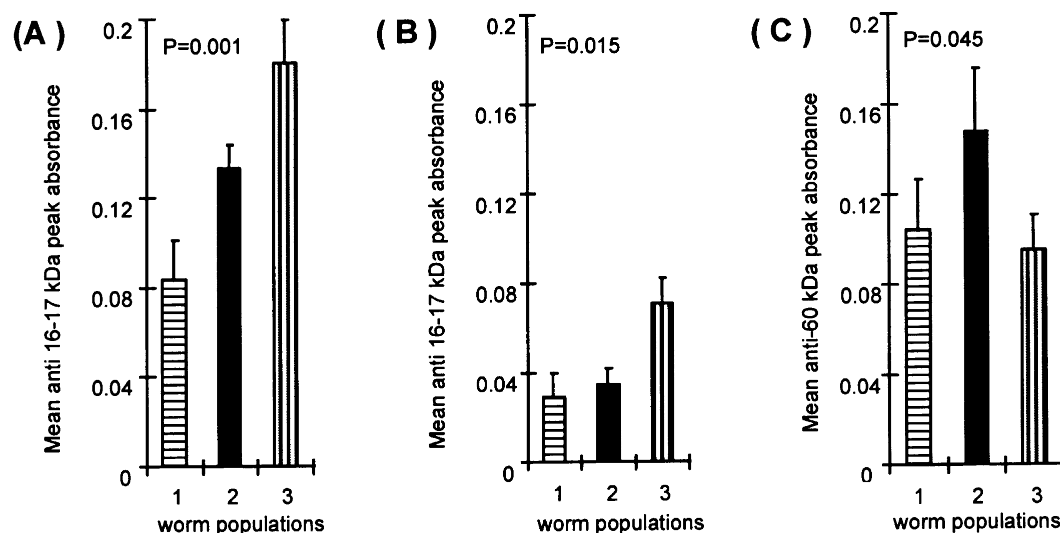

Fig. 3. Quantitative analysis of immunoblot data: comparison of mean recognition of the 16–17 kDa antigen by (A) plasma A and (B) plasma B between the 3 worm populations, (C) mean recognition of the 60 kDa antigen by plasma A. (Data for plasma B were not available.) Vertical lines represent the variance of the mean values.

1/200 in 5% FCS/TBS. Following washing for 1 h with 3 changes of TBS, the NCP was incubated for 3 h with a 1/2000 dilution of peroxidase-labelled anti-human IgG (Dako, UK). Finally, the NCP was washed extensively as before, rinsed for 5 min in PBS and developed with 3,3′-diaminobenzidine (Sigma) diluted in 100 mM Tris–HCl, pH 8/H_2O_2.

Isotype responses to separated proteins were quantified by scanning densitometry analysis, using the 'Discovery Series' desktop scanner (Pharmacia, UK). Responses were analysed and expressed in terms of peak optimal density (peak O.D.) i.e. the highest average pixel value that contributed to the band quantitation.

For each blot, antibody responses to 2 bands were chosen for quantitative analysis. Selection was based on the identification of those bands showing heterogeneity between individual worms. In addition, the decision to quantify responses to the low molecular weight 16–17 kDa antigen was based on the observed prominence of this protein following separation by SDS–PAGE, and also on results of separate studies by Needham *et al*. (1993) and Lillywhite *et al*. (1995) which found IgG subclass responses to a 16–17 kDa antigen to show marked age dependency. Clearly, this analysis is not exhaustive but demonstrates some of the major features of heterogeneity in IgG recognition of individual *T. trichiura* adult worm antigens.

Western blots were run under identical conditions and positive and negative controls were included on each blot. To validate comparison between blots, the background was subtracted from each blot and a correction factor calculated from the reference positive lanes applied to all data. The application of a correction factor did not alter the statistical outcome. However, corrected values only are presented here.

Statistical analysis

Data were analysed using the SPSS and SAS statistical packages. Non-parametric procedures were used throughout due to the non-normal distribution of the data. Analyses of variance with replication (ANOVA) was used to examine differences in antigen recognition of the 3 worm populations. The Mann–Whitney test was used to assess differences in antibody responses to male and female worms, and Spearman rank test was used to test associations between amount of antigen expression and corresponding intensity of antigen recognition by IgG.

RESULTS

Antigen recognition profiles of individual worms

Antigen recognition profiles of the 3 worm populations by IgG antibodies of plasmas A and B are presented in Figs 1 (A–C) and 2 (A–C), respectively.

Comparison of antigenic variability among host worm populations

Mean recognition of the 16–17 kDa antigen is greater for plasma A than for plasma B, however, for recognition by both plasma A and B, mean anti-16–17 kDa responses are highest for population 3 and lowest for population 1 (Fig. 3 A and B). This difference was found to be significant among worm populations for both plasma A ($P = 0{\cdot}001$, Fig. 3 (A) and plasma B ($P = 0{\cdot}015$, Fig. 3 (B)). Mean recognition of the 60 kDa antigen was greatest for worm population 2, with a significant difference in mean recognition observed among the 3 worm populations ($P = 0{\cdot}045$, Fig. 3 (C)).

https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press

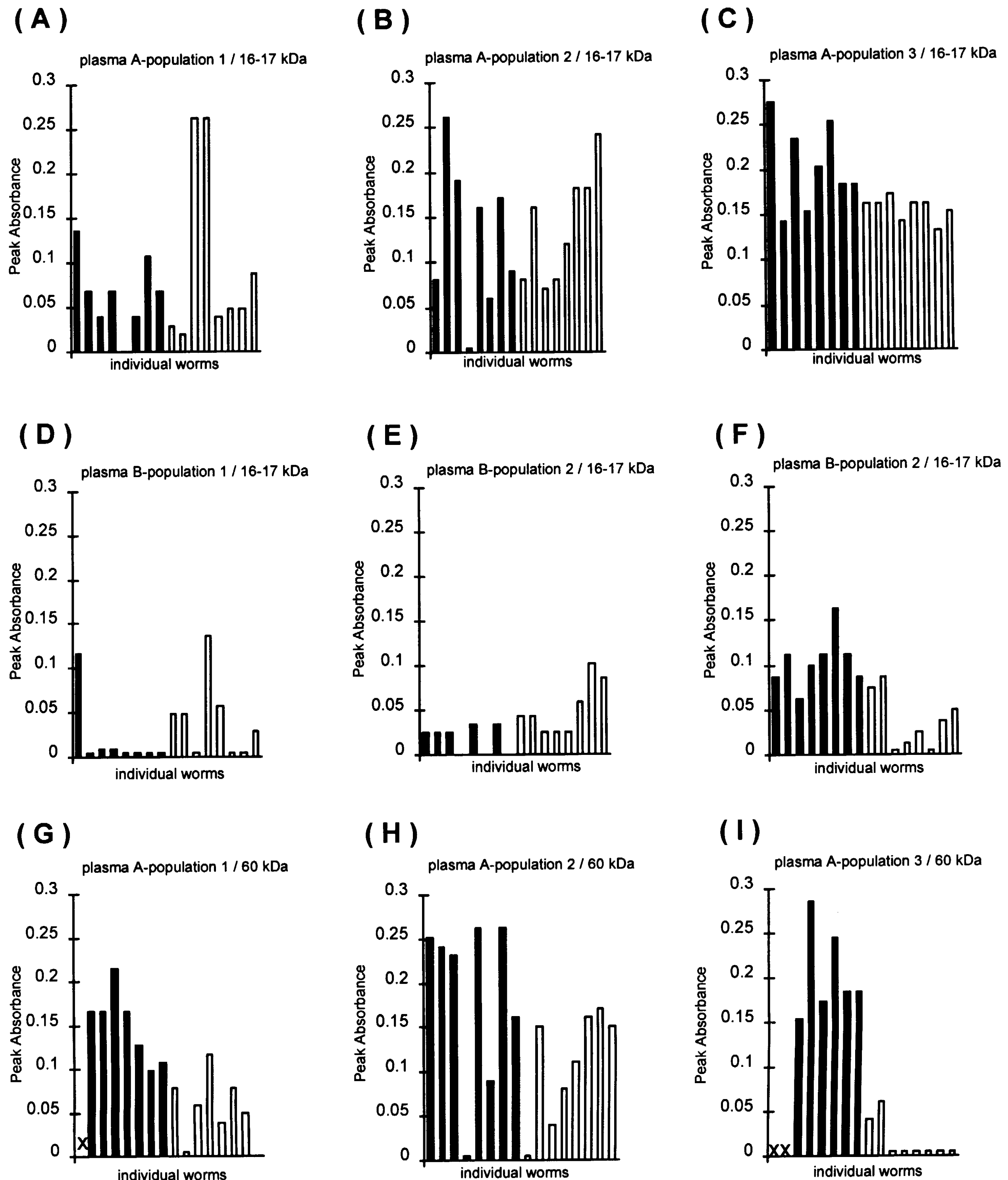

Fig. 4. Heterogeneity in recognition of the 16–17 kDa antigen of within-host worms from populations 1, 2 and 3 by plasma A (A–C), plasma B (D–F) and recognition of the 60 kDa antigen by plasma A (G–I). Male and female worms are represented by dark and light columns, respectively. Where there are no data available, this has been indicated by an '**X**'.

Comparison of antigenic variability in individual worms within the 3 hosts

Intensity of recognition of the 16–17 kDa and 60 kDa antigens by both plasma samples is seen to vary considerably for intra-host worms (Fig. 4).

Comparison of antigenic variability in male and female worms within and among hosts

Significant differences in recognition of the 16–17 kDa protein of male and female worms from worm population 3 were found for both plasma

https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press

Table 1. Comparison of antigen recognition in male and female worms. (A) Recognition of 16–17 kDa by plasma A and plasma B. (B) Recognition of 60 kDa by plasma A

(The standard deviations are given in parentheses; *Z* is the normal approximation to the Mann–Whitney *U* statistic (corrected for ties); *P* is the level of significance.)

Plasma	Worm population	Mean density (S.D.)		*Z*	*P*
		Male worms	Female worms		
(A)					
A	1	0·067	0·100	0·000	1·000
(*n* = 48)		(0·040)	(0·103)		
	2	0·127	0·140	−0·264	0·798
		(0·084)	(0·061)		
	3	0·204	0·157	−2·121	0·038
		(0·048)	(0·013)		
Plasma A total		0·133	0·132	−0·031	0·975
		(0·081)	(0·071)		
B	1	0·018	0·040	−0·634	0·526
(*n* = 48)		(0·040)	(0·045)		
	2	0·018	0·051	−1·837	0·066
		(0·015)	(0·029)		
	3	0·105	0·036	−3·066	0·002
		(0·029)	(0·033)		
Plasma B total		0·047	0·043	−0·269	0·788
		(0·051)	(0·035)		
Total		0·090	0·073	−0·106	0·915
		(0·080)	(0·071)		
(B)					
A	1	0·149	0·060	−2·891	0·004
n = 45		(0·041)	(0·037)		
	2	0·187	0·108	−1·896	0·058
		(0·097)	(0·063)		
	3	0·203	0·013	−0·317	0·002
		(0·050)	(0·024)		
Total		0·179	0·060	−4·562	0·000
		(0·070)	(0·059)		

samples (Table 1.A). Comparison of male and female worms from all 3 populations do not show significant differences in 16–17 kDa recognition for either plasma sample. Recognition of the 60 kDa antigen, however, shows significant differences between male and female worms (Table 1.B).

DISCUSSION

This study has demonstrated heterogeneity in recognition of *T. trichiura* adult worm antigens between 2 infected hosts and has also demonstrated considerable variability in recognition of individual *T. trichiura* adult worm antigens from worms within and among 3 hosts. The former observation is not surprising – host heterogeneity in antigen recognition is well documented and is likely to be influenced by genetic, nutritional and behavioural components (Wakelin, 1988; Bundy, 1988; Bundy & Medley, 1992). However, analyses of antigenic variation within helminth populations have been few. This is the first study to present evidence for antigenic variation within a *T. trichiura* adult worm population.

Antigenic variability in intestinal nematodes has previously been demonstrated by Fraser & Kennedy (1991). Immunofluorescence was used to examine differences in the binding of antibody to the surfaces of individual infective stage larvae of *Ascaris lumbricoides*. Further to considerable heterogeneity in the degree to which individual serum sample antibodies bound to the larva, recognition of individual worms by antibodies from a given individual revealed heterogeneity in surface epitope expression – individual larvae ranged in fluorescence from very bright to negative when incubated with antibodies from 1 plasma sample. The *A. lumbricoides* eggs were embryonated for the same period of time and all hatched and placed into culture simultaneously; therefore this intra-specific variability could not be due to metabolic differences between the larvae.

Results from studies on *Heligmosomoides poly-*

https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press

gyrus, Trichinella spiralis and *Trichuris muris* in mice (Bellaby, Robinson & Wakelin, 1996; Bellaby *et al*. 1995; Bolas-Fernandez & Wakelin, 1989, 1990, 1992; Dobson & Tang, 1991; Goyal, Hermanek & Wakelin, 1994; Goyal & Wakelin, 1993*a, b*; Tang, Dobson & McManus, 1995; Wakelin & Goyal, 1996) and *Strongyloides ratti* in rats (reviewed by Read & Viney, 1996), suggest that parasite populations are not uniformly susceptible to host immune responses. Examination of isolate-specific responses and differences in infection kinetics among isolates have allowed investigation into the role of parasite variables in determining the outcome of infection. Such studies examined variability among parasite isolates in infections of laboratory animals. Under these conditions infections are administered as one dose therefore resulting in all worms being of the same age.

The ages of the *T. trichiura* worms in this study are unknown, as worm burdens build up over a period of years due to repeated exposure to infection. It is possible that the antigenic variability reflects differences in age of the adult worms, or that the variability displayed may represent differences between L4 larvae and adult worms. Although this possibility cannot be discounted, it is unlikely that the worms examined here were not adult worms given the fact that *T. muris* are described as L4 for only 6 days (Panesar, 1989) – a very short period of time in relation to the life-span of the adult worm. A possible explanation for the antigenic variation displayed could be the existence of polymorphic surface antigens as has been shown for *O. lienalis* (Bianco *et al*. 1990). Differential gene expression is a further possibility. Whether this can occur in the expression of antigens by parasitic nematodes is not yet known; however, Poliz, Chin & Herman (1987) have demonstrated incomplete expression of surface-exposed epitopes of the free-living nematode *Caenorhabditis elegans*. Furthermore, the variability in worm recognition is unlikely to result from the drug-induced collection procedure. A recent study found no variability in protein expression between individual *T. muris* worms after oxantel-expulsion from mice (Currie *et al*., in preparation). This is the first study to examine how the expression of antigens varies among nematode populations acquired under natural conditions. Presented here is strong evidence for parasite heterogeneity in natural infections.

In this study, worm populations and the plasma used came from different individuals. It would be interesting to investigate whether plasma from the host that provided the worms gives a similar degree of recognition. Unfortunately, in this study, samples were limited and we were unable to examine this. However, preliminary investigations do suggest the existence of differential recognition of worm antigens by their host and this is currently being investigated further.

It is perhaps surprising that the differences between individual worms may be greater than sex-related differences and differences among hosts. The only significant sex-related difference is the recognition of the 60 kDa antigen – this must be a male-related protein. To date, helminth immuno-epidemiological studies have focused on processes occurring at the individual host level. Current studies of protozoan parasites recognize the importance of parasite variability and future helminth immunological and epidemiological studies must also take this phenomena into account if the processes involved in determining why the size of worm burdens differs between individuals are to be understood.

It is clear from the results presented here that individual *T. trichiura* worms vary quantitatively in the extent to which antibody responses are directed against them. Understanding parasite variability is essential if the complex and often controversial relationship between parasite and host occurrence is to be understood. Accurate parasite characterization and the application of appropriate molecular techniques to studies on parasite transmission will help to answer fundamental questions about host risk factors. A perception of the processes involved in such variability will not only contribute to explaining heterogeneity in susceptibility and pathology but will also be relevant to vaccination. Whatever the cause of the observed heterogeneity within *T. trichiura* adult worm populations, the consequences are undoubtedly of value to a parasite population facing host heterogeneity in immune responsiveness and in acquired immunity due to previous exposure.

The authors would like to thank Jane Lillywhite, Guy Barker, Edwin Michael and Christl Donnelly for helpful discussions. This work was supported by the Wellcome Trust and the Medical Research Council. R.M.C. is supported by an MRC studentship.

REFERENCES

BELLABY, T., ROBINSON, K. & WAKELIN, D. (1996). Induction of differential T-helper-cell responses in mice infected with variants of the parasitic nematode *Trichuris muris*. *Infection and Immunity* **64**, 791–795.

BELLABY, T., ROBINSON, K., WAKELIN, D. & BEHNKE, J. M. (1995). Isolates of *Trichuris muris* vary in their ability to elicit protective immune responses to infection in mice. *Parasitology* **111**, 353–357.

BIANCO, A. E., ROBERTSON, B. D., KUO, Y-M., TOWNSON, S. & HAM, P. J. (1990). Developmentally regulated expression and secretion of a polymorphic antigen by *Onchocerca* infective-stage larvae. *Molecular and Biochemical Parasitology* **39**, 203–212.

BOLAS-FERNANDEZ, F. & WAKELIN, D. (1989). Infectivity of *Trichinella* isolates in mice is determined by host immune responsiveness. *Parasitology* **99**, 83–88.

BOLAS-FERNANDEZ, F. & WAKELIN, D. (1990). Infectivity, antigenicity and host responses to isolates of the genus *Trichinella*. *Parasitology* **100**, 491–498.

https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press

BOLAS-FERNANDEZ, F. & WAKELIN, D. (1992). Immunization against geographical isolates of *Trichinella spiralis* in mice. *International Journal for Parasitology* **22**, 773–781.

BUNDY, D. A. P. (1988). Population ecology of intestinal helminth infections in human communities. *Philosophical Transactions of the Royal Society of London, B* **321**, 405–420.

BUNDY, D. A. P. & MEDLEY, G. F. (1992). Immunoepidemiology of human geohelminthiasis: ecological and immunological determinants of worm burden. *Parasitology* **104**, 105–119.

DOBSON, C. & TANG, J-M. (1991). Genetic variation and host–parasite relations: *Nematospiroides dubius* in mice. *Journal of Parasitology* **77**, 884–889.

FRASER, E. M. & KENNEDY, M. R. (1991). Heterogeneity in the expression of surface-exposed epitopes among the larvae of *Ascaris lumbricoides*. *Parasite Immunology* **13**, 219–225.

GOYAL, P. K., HERMANEK, J. & WAKELIN, D. (1994). Lymphocyte proliferation and cytokine production in mice-infected with different geographical isolates of *Trichinella spiralis*. *Parasite Immunology* **16**, 105–110.

GOYAL, P. K. & WAKELIN, D. (1993 *a*). Influence of variation in host strain and parasite isolate on inflammatory and antibody responses to *Trichinella spiralis* in mice. *Parasitology* **106**, 371–378.

GOYAL, P. K. & WAKELIN, D. (1993 *b*). Vaccination against *Trichinella spiralis* in mice using antigens from different isolates. *Parasitology* **107**, 311–317.

HASWELL-ELKINS, M. R., KENNEDY, M. W., MAIZELS, R. M., ELKINS, D. B. & ANDERSON, R. M. (1989). The antibody recognition profiles of humans naturally infected with *Ascaris lumbricoides*. *Parasite Immunology* **11**, 615–627.

HASWELL-ELKINS, M. R., KENNEDY, M. W., MAIZELS, R. M., ELKINS, D. B. & ANDERSON, R. M. (1992). Immunoepidemiology of *Ascaris lumbricoides*: relationships between antibody specificities, exposure and infection in a human community. *Parasitology* **104**, 153–159.

KENNEDY, M. W., TOMLINSON, L. A., FRASER, E. M. & CHRISTIE, J. F. (1990). The specificity of the antibody response to internal antigens of *Ascaris*: heterogeneity in infected humans, and MHC (H-2) control of the repertoire in mice. *Clinical and Experimental Immunology* **80**, 219–224.

LILLYWHITE, J. E., COOPER, E. S., NEEDHAM, C. S., VENUGOPAL, S., BUNDY, D. A. P. & BIANCO, A. E. (1995). Identification and characterisation of excreted/secreted products of *Trichuris trichiura*. *Parasite Immunology* **17**, 47–54.

NEEDHAM, C. S., LILLYWHITE, J. E., DIDIER, J. M., BIANCO, A. E. & BUNDY, D. A. P. (1993). Age-dependency of serum isotype responses and antigen recognition in human whipworm (*Trichuris trichiura*) infection. *Parasite Immunology* **15**, 683–692.

PANESAR, T. S. (1989). The molting pattern in *Trichuris muris* (Nematoda: Trichuroidea). *Canadian Journal of Zoology* **67**, 2340–2343.

POLITZ, S. M., CHIN, K. J. & HERMAN, D. L. (1987). Genetic analysis of adult-specific surface antigenic differences between varieties of the nematode *Caenorhabditis elegans*. *Genetics* **117**, 467–476.

READ, A. F. & VINEY, M. E. (1996). Helminth immunogenetics: why bother? *Parasitology Today* **12**, 337–343.

TANG, J-M., DOBSON, D. & McMANUS, D. P. (1995). Antigens in phenotypes of *Heligmosomoides polygyrus* raised selectively from different strains of mice. *International Journal for Parasitology* **25**, 847–852.

WAKELIN, D. (1988). Helminth infections. In *Genetics of Resistance to Bacterial and Parasitic Infection* (ed. Wakelin, D. & Blackwell, J. M.), pp. 153–224. Taylor and Francis, London.

WAKELIN, D. & GOYAL, P. K. (1996). *Trichinella* isolates: Parasite variability and host responses. *International Journal for Parasitology* **26**, 471–481.

https://doi.org/10.1017/S0031182098003084 Published online by Cambridge University Press