Methods for estimation of associations between multiple species parasite infections

S. C. HOWARD*, C. A. DONNELLY *and* M.-S. CHAN

Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3FY

(*Received 27 May 2000; revised 20 July 2000; accepted 20 July 2000*)

SUMMARY

Human populations are often infected with more than one species of parasite, especially in developing countries where overall rates of parasitism are high. Infections with multiple parasite species may not necessarily be independent within an individual as physiological, immunological or ecological factors may result in positive or negative associations between infections with different parasite species. A general framework for estimation of these associations is presented. Data from over 215000 individuals are analysed and the associations between geohelminth (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) and malaria species are investigated. A method is presented for analysing data from multiple communities and testing whether the associations in different communities are equal. Overall estimates of the associations between species are obtained for each country and continent where data were available. Associations between geohelminth species were, in general, found to be positive whilst both positive and negative associations were found between the different *Plasmodium* species. There was evidence for significant geographical heterogeneity between the associations. A method for using these parameter estimates to predict the distribution of multiple infections when only marginal prevalence data are available is described and demonstrated.

Key words: multiple species, log-linear, geohelminths, *Plasmodium* spp.

INTRODUCTION

Simultaneous investigations of multiple parasite species have often found that single communities support a variety of parasites (e.g. Petney & Andrews, 1998), especially in developing countries, where overall rates of parasitism are high. This may lead to increased risks of morbidity and mortality for those individuals who harbour multiple infections. To maximize available resources it may be advantageous to consider the impacts of multiple species control programmes (Bundy *et al*. 1991). The successful implementation of such programmes will require knowledge (or estimation) of the pattern of multiple infections within individuals.

Studies of multiple parasite species infections appear in a variety of forms. With prevalence data from a number of different communities it is possible to calculate correlations between the prevalence of different species (e.g. Booth & Bundy, 1992; Jemaneh, 1998; Brooker, Booth & Guyatt, 1999*a*). Such studies provide information as to associations between the species on a community level. Another possibility is to consider the correlation between the intensities of the different species infections within individual hosts (e.g. Haswell-Elkins, Elkins & Anderson, 1987; Holland *et al*. 1989). Thirdly, it is possible to cross-classify individuals within a community according to their infection status for each of the parasite species present in the community; this results in frequency data for the number of individuals with each of the possible infection combinations. It is this latter form of data, which provides insights into associations within individual hosts, that is analysed here.

In this paper we concentrate primarily on 2 groups of parasite infections. The first of these are intestinal helminth infections, for which the study of multiple infections is particularly relevant. There are approximately 1 billion people worldwide infected with each of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm, nearly all of them being in the developing countries and mostly in children (Chan *et al*. 1994). Results from a parasite survey of nearly 1·5 million people in China indicated that among China's population of 1·13 billion people there are 531 million people infected with *A. lumbricoides*, 212 million infected with *T. trichiura* and 194 million infected with hookworm (Hotez *et al*. 1997). The proportions of the world population infected with these parasites remains virtually unchanged over the last 50 years and the actual numbers infected have increased greatly (Chan, 1997).

The second type of multiple infections considered are those concerning the 4 *Plasmodium* species that cause human malaria: *P. falciparum*, *P. malariae*, *P.*

* Corresponding author: Stroke Prevention Unit, Department of Clinical Neurology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE. Tel: +01865 224152.
E-mail: sally.howard@clinical-neurology.oxford.ac.uk

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

ovale and *P. vivax*. The most pathogenic of the 4 species, *P. falciparum*, is frequently found to occur with *P. vivax* or *P. malariae*, and in some situations infections with all 4 species have been found in the same host (Purnomo *et al.* 1999; Snounou *et al.* 1993; Zhou *et al.* 1998). Richie (1988) reported a study that found at least 61·5% of a group of children to harbour infections with more than 1 species. A number of historical and recent discussions of the associations between species have been published (Knowles & White, 1930; Cohen, 1973; Molineaux *et al.* 1980; Richie, 1988; McKenzie & Bossert, 1997, 1999), with mixed conclusions as to the nature of the associations.

The methods presented in this paper extend previous uses of log-linear models to investigate associations between species (Cohen, 1973; Salem *et al.* 1994; Booth & Bundy, 1995; Booth, Li & Tanner, 1996; Booth *et al.* 1998*a*; Booth, Mayombana & Kilima, 1998*b*; Needham *et al.* 1998; McKenzie & Bossert, 1997, 1999; Brooker *et al.* 2000). A structure is provided for the examination of all possible associations within individuals between an arbitrary number of parasite species for a single population or multiple populations. Furthermore, a basis is defined for testing whether the associations between parasite species are equal in different communities. This provides a way of assessing whether there is geographical heterogeneity amongst the associations between different species.

A novel use of this model in parasite epidemiology is the prediction of the number of multiple infections in a population when only the marginal prevalence of each parasite species is available. In calculating the global burden of parasite infections, the positive associations within individuals are critical because the assumption of independence between parasite infections can lead to the underestimation of the proportion of the population experiencing multiple infection. The parameter estimates obtained can be used with an iterative procedure to estimate the number of double and triple infections in a new population for which only the marginal prevalences of each species are known. This procedure is demonstrated for datasets where only the marginal totals are available and the results compared to those that would have been obtained under the assumption of independence.

MATERIALS AND METHODS

The data

Survey data on the joint distribution of 3 helminths, *A. lumbricoides*, *T. trichiura* and hookworm, were analysed from 44 populations consisting of more than 52000 children and 23 mixed-age populations consisting of over 16000 adults and children. Within each of these datasets infections with each of the 2 main human hookworm species (*Necator americanus* and *Ancyclostoma duodenale*) are treated as a single type of infection. In some cases, infections with other species were reported; however, individuals were simply classified according to their status with each of the 3 helminths considered.

Data on the joint distribution on 2 or more of the 4 malaria species were analysed from 26 populations consisting of 13000 children and 48 populations consisting of over 114000 adults and children.

The data sets obtained are listed in Table 1, together with the country of survey, sample size, age of study population and species present. In addition to the datasets concerning geohelminth and malaria infections, some data on multiple infections with the 2 hookworm species and 2 schistosome species (*S. haematobium* and *S. mansoni*) were analysed.

THE MODEL

Frequency data on the numbers of single and multiple infections within a population can be represented in a contingency table and analysed using log-linear models (Fienberg, 1970; Everitt, 1977). For each population, the probability of infection with single and multiple parasite species can be modelled using log-linear regression. The log-linear regression equations contain parameters corresponding to associations, which may be estimated by maximum likelihood methods. Hypotheses, such as the equality of associations in multiple populations, may be tested using likelihood ratio tests. It should be noted that where the term association (or interaction) is used, this refers to a statistical association between the frequencies, as distinct from a biological interaction between the parasite species involved. It is this former type of association that is the subject of the analysis.

Consider the joint distribution of infections with 3 parasites within each of M populations indexed by m where $m = 1, \ldots, M$. Let I_i, I_j and I_k represent the infection status for each of these 3 parasites, i, j and k, such that $I_i = 1$ if an individual is infected with parasite i and $I_i = 0$ if the individual is not infected with parasite i, and so on. Let $f_{m:I_iI_jI_k}$ be the number of individuals in each of the 8 possible infection categories in population m. Thus, $f_{m:000}$ is the number of individuals harbouring no infections in population m and $f_{m:111}$ is the number in the same population who are infected with all 3 parasite species.

The log-linear model including 2- and 3-way associations is of the form:

$$\log(f_{m:I_iI_jI_k}) = \mu_m + \alpha_{m:i}I_i + \alpha_{m:j}I_j + \alpha_{m:k}I_k + \beta_{m:ij}I_iI_j + \beta_{m:ik}I_iI_k + \beta_{m:jk}I_jI_k + \beta_{m:ijk}I_iI_jI_k,$$

where I_j equals 1 if infected with parasite j and 0 otherwise.

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

Table 1. Summary characteristics of all data sets analysed

(Symbols used to denote different parasite species reported are as follows: F = *Plasmodium falciparum*, M = *Plasmodium malariae*, O = *Plasmodium ovale*, V = *Plasmodium vivax*, A = *Ascaris lumbricoides*, T = *Trichuris trichiura*, H = *Hookworm*, N = *Necator americanus*, D = *Ancylostome duodenale*, Sh = *Schistosoma haematobium*, Sm = *Schistosoma mansoni*. $^{+}$ indicates a positive association between the species and $^{-}$ indicates a negative association. Mixed ages indicates that the data were collected amongst both adults and children or adults only. * See Knowles & White (1930).)

No.	Reference	Country	*n*	Ages	Species	Sig. associations
1	Alonzo *et al.* (1993)	Benin	18512	Children	ATH	AT^{+}, AH^{-}, TH^{+}, ATH^{+}
2	Daubney & Carman (1928)	Kenya	152	Children	ATH	TH^{+}
3	Kinoti (1971)	Kenya	740	Children	ATH	—
4	Brooker *et al.* (1999*b*)	Kenya	460	Children	ATH	AT^{+}, TH^{+}
5	Kightlinger *et al.* (1995)	Madagascar	272	Children	ATH	AH^{+}, TH^{+}
6	Kightlinger *et al.* (1995)	Madagascar	272	Children	ATH	AH^{+}, TH^{+}
7	March'Hadour *et al.* (1960)	Madagascar	254	Children	ATH	—
8	Mafiana *et al.* (1998)	Nigeria	1060	Children	ATH	AT^{+}, AH^{+}, TH^{+}, ATH^{-}
9	Ilardi *et al.* (1985)	Somalia	158	Children	ATH	TH^{-}
10	Booth *et al.* (1998*a*)	Tanzania	1539	Children	ATH	AT^{+}, AH^{+}, TH^{+}
11	Meakins *et al.* (1981)	Tanzania	185	Children	AH	—
12	Ismid *et al.* (1981)	Indonesia	61	Children	ATH	AT^{+}, AH^{+}, TH^{+}
13	Ismid *et al.* (1981)	Indonesia	50	Children	ATH	AT^{+}, AH^{+}, TH^{+}
14	Ismid *et al.* (1981)	Indonesia	47	Children	ATH	—
15	Kan, 1984	Malaysia	232	Children	ATH	AT^{+}, AH^{+}
16	Kan, 1984	Malaysia	95	Children	ATH	—
17	Kan, 1984	Malaysia	97	Children	ATH	—
18	Kan, 1984	Malaysia	123	Children	ATH	AT^{-}
19	Kan, 1984	Malaysia	296	Children	ATH	AT^{+}
20	Kan, 1984	Malaysia	219	Children	ATH	AT^{+}
21	Kan, 1984	Malaysia	49	Children	ATH	AH^{+}
22	Kan, 1984	Malaysia	46	Children	ATH	—
23	Kan, 1986	Malaysia	318	Children	AT	—
24	Kan, 1986	Malaysia	170	Children	ATH	—
25	De Silva & Jayatilleka (1981)	Sri Lanka	100	Children	ATH	—
26	Chen (1964)	Taiwan	937	Children	ATH	AT^{+}, AH^{+}, TH^{+}
27	Yokogawa *et al.* (1983)	Thailand	334	Children	ATH	AH^{+}, TH^{+}
28	Yokogawa *et al.* (1983)	Thailand	323	Children	ATH	—
29	Yokogawa *et al.* (1983)	Thailand	111	Children	ATH	—
30	Upatham *et al.* (1989)	Thailand	518	Children	ATH	AT^{+}
31	Toan (1993)	Vietnam	2331	Children	ATH	AT^{+}, AH^{+}, TH^{-}
32	Toan (1993)	Vietnam	3083	Children	ATH	AT^{+}, AH^{+}, TH^{+}
33	Toan (1993)	Vietnam	3096	Children	ATH	AT^{-}, AH^{-}, TH^{+}
34	Toan (1993)	Vietnam	3028	Children	ATH	AT^{+}, AH^{+}, TH^{+}
35	Toan (1993)	Vietnam	3174	Children	ATH	TH^{+}
36	Toan (1993)	Vietnam	5802	Children	ATH	AH^{-}
37	Toan (1993)	Vietnam	2488	Children	ATH	AH^{-}
38	Needham *et al.* (1998)	Vietnam	543	Children	ATH	AT^{+}
39	Dazo (1989)	Papua New Guinea	240	Children	ATH	—
40	Fulmer & Huempfner (1965)	USA	52	Children	ATH	AT^{+}
41	Fulmer & Huempfner (1965)	USA	186	Children	ATH	AT^{+}, AH^{+}
42	Fulmer & Huempfner (1965)	USA	73	Children	ATH	AT^{+}
43	Fulmer & Huempfner (1965)	USA	180	Children	ATH	AT^{+}
44	Saldiva *et al.* (1999)	Brazil	520	Children	AT	AT^{+}
45	Meunier *et al.* (1984)	Central African Rep.	3352	Mixed	ATH	TH^{+}
46	Stürchler *et al.* (1980)	Liberia	690	Mixed	ATH	AT^{+}, AH^{+}, TH^{+}, ATH^{-}
47	Barbier *et al.* (1975)	Madagascar	261	Mixed	ATH	AT^{+}, AH^{+}, TH^{+}, ATH^{-}
48	Adeyeba & Dipeolu (1984)	Nigeria	283	Mixed	ATH	AT^{+}, AH^{+}
49	Onadeko & Ladipo (1989)	Nigeria	827	Mixed	ATH	AT^{+}, AH^{+}, TH^{+}, ATH^{-}
50	Udonsi *et al.* (1996)	Nigeria	300	Mixed	ATH	AT^{+}, AH^{+}, TH^{+}
51	Ilardi *et al.* (1980)	Somalia	142	Mixed	ATH	AT^{+}
52	Jancloes & Cornet (1980)	Zaire	3056	Mixed	ATH	AT^{+}, AH^{+}
53	Booth *et al.* (1996)	China	469	Mixed	AT	AT^{+}
54	Booth *et al.* (1996)	China	377	Mixed	AT	—
55	Booth *et al.* (1996)	China	430	Mixed	AT	—
56	Dhanachand & Anand (1997)	India	300	Mixed	ATH	—
57	Higgins *et al.* (1984)	Indonesia	227	Mixed	ATH	AT^{+}, AH^{+}, TH^{+}
58	Higgins *et al.* (1984)	Indonesia	831	Mixed	ATH	AT^{+}, AH^{+}

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

Table 1 (cont.)

No.	Reference	Country	*n*	Ages	Species	Sig. associations
59	Higgins *et al.* (1984)	Indonesia	329	Mixed	ATH	—
60	Cabrera *et al.* (1989*a*)	The Philippines	1182	Mixed	ATH	AT^+
61	Cabrera *et al.* (1989*b*)	The Philippines	569	Mixed	ATH	AT^+, AH^+, TH^+
62	Kan (1993)	Malaysia	319	Mixed	ATH	AT^+
63	Sinniah *et al.* (1978)	Malaysia	150	Mixed	ATH	AT^+
64	Upatham *et al.* (1989)	Thailand	1142	Mixed	ATH	AT^+, AH^+, TH^+
65	Dazo (1989)	Samoa	224	Mixed	ATH	AT^+, TH^+
66	Dazo (1989)	Vanuatu	457	Mixed	ATH	AT^+, TH^+
67	Ferreira *et al.* (1994)	Brazil	407	Mixed	AT	AT^+
68	Campbell *et al.* (1987)	Kenya	147	Children	FMO	FM^+, MO^+
69	Campbell *et al.* (1987)	Kenya	142	Children	FMO	FM^+, MO^-
70	Campbell *et al.* (1987)	Kenya	196	Children	FMO	FM^+, MO^+
71	May *et al.* (1999)	Nigeria	230	Children	FMO	FM^+, MO^+
72	May *et al.* (1999)	Nigeria	59	Children	FMO	—
73	May *et al.* (1999)	Nigeria	142	Children	FM	—
74	Leger *et al.* (1923)	Nigeria	250	Children	FMV	FM^-
75	Bedier *et al.* (1924)	Nigeria	135	Children	FMV	FM^-
76	Knowles & White (1930)	Sierra Leone	809	Children	FMV	FV^+, MV^+
77	Alifrangis *et al.* (1999)	Tanzania	126	Children	FMO	—
78	Hellgren *et al.* (1994)	Tanzania	163	Children	FMO	—
79	Thomson *et al.* (1994)	The Gambia	1465	Children	FMO	FM^+
80	Gbary *et al.* (1988)	Togo	707	Children	FMO	FM^+, FO^+
81	Brown *et al.* (1970)	Uganda	2899	Children	FM	FM^+
82	Dorolle (1927)	China	643	Children	FMV	FV^-, MV^+
83	Perry (1911)	India	351	Children	FMV	—
84	Phillips (1923)*	India	645	Children	FMV	FV^-
85	Aiyer (1924)*	India	57	Children	FMV	—
86	Roy (1926)*	India	309	Children	FV	—
87	Christophers & Shortt (1921)	Iraq	76	Children	FMV	—
88	Lalor (1913)*	Myanmar	207	Children	FMV	FV^-
89	Strickland *et al.* (1988)	Pakistan	2891	Children	FV	—
90	Maitland *et al.* (1996)	Vanuatu	292	Children	FV	—
91	Maitland *et al.* (1996)	Vanuatu	211	Children	FV	FV^-
92	Maitland *et al.* (1996)	Vanuatu	302	Children	FV	FV^-
93	Wilding *et al.* (1995)	Gabon	2192	Mixed	FMO	—
94	Landgraf *et al.* (1994)	Ghana	1048	Mixed	FMO	FM^-
95	Deloron *et al.* (1989)	Kenya	222	Mixed	FMO	—
96	Deloron *et al.* (1989)	Kenya	245	Mixed	FMO	—
97	Deloron *et al.* (1989)	Kenya	253	Mixed	FMO	FM^+
98	Deloron *et al.* (1989)	Kenya	225	Mixed	FMO	—
99	May *et al.* (1999)	Nigeria	159	Mixed	FMO	—
100	Trape *et al.* (1992)	Senegal	2465	Mixed	FMO	FM^+
101	Trape *et al.* (1994)	Senegal	8539	Mixed	FMO	FM^+, FO^+, MO^+
102	Molineaux *et al.* (1980)	Sudan	7026	Mixed	FMO	FM^+, FO^+, MO^+
103	Molineaux *et al.* (1980)	Sudan	6526	Mixed	FMO	FM^+, FO^+, MO^+
104	Nhonoli *et al.* (1974)	Tanzania	360	Mixed	FM	—
105	Nhonoli *et al.* (1974)	Tanzania	450	Mixed	FMV	FM^+
106	Wilson (1936)	Tanzania	3393	Mixed	FMV	FM^+, FV^+, MV^+
107	Rosenberg & Maheswary (1982)	Bangladesh	1093	Mixed	FV	FV^-
108	Borel & Levanan (1927)	China	1253	Mixed	FMV	—
109	Borel & Levanan (1927)	China	1024	Mixed	FMV	FV^+
110	Stocker (1923)*	India	412	Mixed	FMV	FV^+
111	Banerjea (1930)*	India	1519	Mixed	FMV	FV^-
112	Khambata (1913)*	India	112	Mixed	FMV	—
113	Ramsay (1928)*	India	1514	Mixed	FMV	FV^-
114	Bailey (1928)*	India	1068	Mixed	FMV	FM^-, FV^-
115	Purnomo *et al.* (1999)	Indonesia	51	Mixed	FMOV	—
116	Masterman (1913)	Israel	700	Mixed	FMV	FM^-, FV^-, MV^-
117	Angus (1919)*	Israel	40168	Mixed	FMV	FV^-
118	Gordon *et al.* (1991)	Malaysia	268	Mixed	FMV	FV^+, MV^+
119	Lalor (1912)*	Myanmar	151	Mixed	FMV	FV^-
120	Strickland *et al.* (1987)	Pakistan	2699	Mixed	FV	FV^+
121	Strickland *et al.* (1987)	Pakistan	2643	Mixed	FMV	—

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

Table 1 (cont.)

No.	Reference	Country	*n*	Ages	Species	Sig. associations
122	Strickland *et al.* (1987)	Pakistan	2699	Mixed	FMV	—
123	Carter (1927)*	Sri Lanka	11260	Mixed	FMV	FM^-, FV^-, MV^-
124	Schuffner (1938)	Sumatra	3266	Mixed	FMV	FV^+, MV^+
125	Snounou *et al.* (1993)	Thailand	196	Mixed	FMOV	—
126	Nosten *et al.* (1991)	Thailand	1358	Mixed	FV	FV^-
127	Banchongaksorn *et al.* (1996)	Thailand	913	Mixed	FMV	FV^-
128	Zhou *et al.* (1998)	Thailand	548	Mixed	FMOV	FM^-, FV^-, MO^+
129	Chardamatis (1911)	Greece	87	Mixed	FMV	FM^-
130	Treadgold (1918)	Macedonia	540	Mixed	FMV	—
131	Yakimoff (1923)	Russia	1638	Mixed	FMV	—
132	Collins *et al.* (1988)	Papua New Guinea	614	Mixed	FMV	FV^+
133	Mizushima *et al.* (1994)	Solomon Islands	506	Mixed	FMV	MV^+
134	Buxton (1927)	Vanuatu	209	Mixed	FV	—
135	von Esdorf (1912)*	USA	87	Mixed	FMV	—
136	Griffitts (1925)	USA	75	Mixed	FMV	FV^+
137	United Fruit Co. (1925)*	Mexico	2742	Mixed	FMV	FV^-
138	Godoy and Pinto (1923)*	Brazil	86	Mixed	FMV	MV^+
139	Sulzer *et al.* (1975)	Peru	122	Mixed	MV	—
140	Sulzer *et al.* (1975)	Peru	125	Mixed	MV	—
141	Carme (1984)	Congo	230	Children	ND	ND^+
142	Hsieh *et al.* (1972)	Liberia	292	Children	ND	—
143	Hsieh *et al.* (1972)	Liberia	628	Children	ND	ND^+
144	Hsieh *et al.* (1972)	Liberia	644	Children	ND	—
145	Hsieh *et al.* (1972)	Liberia	446	Children	ND	—
146	Hsieh *et al.* (1972)	Liberia	1165	Children	ND	ND^+
147	Hsieh *et al.* (1972)	Liberia	1058	Children	ND	—
148	Udonsi (1984)	Nigeria	1623	Mixed	ND	ND^+
149	Sturrock (1966*a*)	Tanzania	391	Mixed	ND	—
150	Sturrock (1966*b*)	Uganda	343	Mixed	ND	ND^+
151	Ahmed *et al.* (1996)	Sudan	902	Children	ShSm	—
152	Ahmed *et al.* (1996)	Sudan	1511	Children	ShSm	$ShSm^+$
153	Ahmed *et al.* (1996)	Sudan	1791	Children	ShSm	$ShSm^+$
154	Ahmed *et al.* (1996)	Sudan	1918	Children	ShSm	$ShSm^+$
155	Lwambo *et al.* (1999)	Tanzania	6897	Children	ShSm	$ShSm^-$
156	Loum (1974)	Tanzania	2498	Mixed	ShSm	$ShSm^+$
157	Chandiwana *et al.* (1989)	Zimbabwe	58	Mixed	ShSm	$ShSm^+$
158	Creasey *et al.* (1982)	Zimbabwe	158	Mixed	ShSm	$ShSm^+$

The inclusion of the β terms representing associations allows for the infection with multiple species to be non-independent. This means that the presence of 1 species influences the likelihood that another species will be present. The parameters are interpreted by considering the odds ratios for infection with each species. This model corresponds to the odds ratios of $e^{\beta_{m:ij}}$, $e^{\beta_{m:ik}}$, $e^{\beta_{m:ij}+\beta_{m:ik}+\beta_{m:ijk}}$ comparing the odds of infection with parasite i in those infected with parasite j only, those infected with parasite k only and those infected with both parasites j and k, respectively, to those not infected by either parasite j or parasite k. Similar interpretations are obtained for the odds of infection with parasites j and k. Thus, a positive β parameter for the association between 2 parasite species indicates that joint infections with those 2 species are more likely than would be expected under the hypothesis of independence.

This model can be easily extended to apply to situations where the joint distribution of more than 3 parasites is of interest, for example multiple species malaria infections. If there are N different parasite species, the model can be expanded to include higher-order interactions up to the N-way interaction. The model containing q-way and lower interactions contains $[\binom{N}{2}+\binom{N}{3}+\cdots+\binom{N}{q}]$ β parameters for each population. Maximum likelihood estimates of the α and β parameters can be obtained from joint distribution data.

Hypothesis tests

Likelihood ratio hypothesis tests may be performed within a single population or comparing multiple populations. Within any population the null hypothesis of independence of infections can be tested. This is equivalent to testing

$$H_{01}: \beta_{m:ij} = \beta_{m:ik} = \beta_{m:jk} = \beta_{m:ijk} = 0.$$

For 3 parasite species, the test of independence of infections has 4 degrees of freedom for each population and thus has $4M$ degrees of freedom when all M populations are tested simultaneously.

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

If the model of independence is not a satisfactory fit, then the most parsimonious model describing the data can be obtained by assessing the inclusion of the β parameters. A step-up model building procedure can be used. For example, in a population with 3 parasite species, each of the 2-way associations can be examined individually assuming the others to be equal to zero; the one which results in the most significant reduction in the residual deviance is retained and then the remaining β terms can be assessed similarly, until the addition of a further term does not result in a significant improvement. This procedure is hierarchical in that a 3-way association is only considered if each of the 2-way associations are significant.

If more than 1 significant association is found, it may be of interest to test whether the different β terms can be assumed to be equal, either within a single population or between multiple populations:

$$H_{02}: \beta_{m:ij} = \beta_{m:ik} = \beta_{m:jk} \quad \forall m.$$

If this hypothesis were true, this could be interpreted as indicating that the presence of infection with species i or j had equal effects upon the probability of concurrent infection with species k and similarly for the other combinations.

For multiple populations, it is possible to test the hypothesis that the β parameters are equal for all M populations. For a model with 2- and 3-way interactions, this hypothesis may be written as:

$$H_{03}: \begin{cases} \beta_{m:ij} = \beta_{ij} \ \forall j \neq i; \\ \beta_{m:ijk} = \beta_{ijk} \ \forall j \neq i;\ k \neq i, j; \end{cases} \quad \forall m,$$

where $i, j, k = 1, \ldots, N$.

For example, to test whether all $\beta_{m:ij}$ could be assumed to be equal within m different communities a model including m separate parameters $\beta_{1:ij}, \beta_{2:ij}, \ldots, \beta_{m:ij}$ can be compared to a model containing a single β_{ij} parameter which was assumed to be equal in each of the m populations. Under the null hypothesis, the difference in residual deviances will follow a chi-squared distribution with $m-1$ degrees of freedom.

Estimation of the joint distribution of infection in a population with known infection prevalences

Given only the marginal prevalence of each species, it is possible to estimate the joint density of parasite infections in a population based on the estimates of the associations between the parasite species. For illustration of this method the number of parasite species present in the population is assumed to be equal to 3. The joint density can be obtained using the following updating estimation procedure.

Define the marginal prevalences of infection with parasites i, j and k in population m by $\pi_{m:i+}$, $\pi_{m:j+}$ and $\pi_{m:k+}$ respectively. Thus $\pi_{m:i+}$ is the proportion of individuals infected with parasite i, either as a single infection or in combination with other infections. Define $\pi_{m:i+j+k+}$ to be the probability of joint infection with parasites i, j and k and $\pi_{m:i+j+k-}$ to be the probability of joint infection with parasites i and j but the absence of infection with parasite k, and so on.

The first stage of the procedure is to calculate the joint probabilities of infection under the assumption that infections with different parasite species are independent. For example, estimate $\pi_{m:i+j+k+}$ by multiplying the unconditional probabilities $\pi_{m:i+}$, $\pi_{m:j+}$ and $\pi_{m:k+}$ and estimate $\pi_{m:i-j+k+}$ by multiplying the unconditional probabilities $(1-\pi_{m:i+})$, $\pi_{m:j+}$ and $\pi_{m:k+}$, and so on.

The estimate for $\alpha_{m:i}$ is obtained by expressing the probability of infection with parasite i in terms of the conditional probabilities of infection with parasite i given the infection status with parasites j and k.

This is equivalent to equating $\pi_{m:i+}$ and

$$\frac{e^{\alpha_{m:i}}}{1+e^{\alpha_{m:i}}}\pi_{m:j-k-} + \frac{e^{\alpha_{m:i}+\beta_{m:ij}}}{1+e^{\alpha_{m:i}+\beta_{m:ij}}}\pi_{m:j+k-} + \frac{e^{\alpha_{m:i}+\beta_{m:ik}}}{1+e^{\alpha_{m:i}+\beta_{m:ik}}}\pi_{m:j-k+} + \frac{e^{\alpha_{m:i}+\beta_{m:ij}+\beta_{m:ik}+\beta_{m:ijk}}}{1+e^{\alpha_{m:i}+\beta_{m:ij}+\beta_{m:ik}+\beta_{m:ijk}}}\pi_{m:j+k+}$$

where $j \neq k \neq i$. $\pi_{m:j-k-}$ is obtained by summing $\pi_{m:i+j-k-}$ and $\pi_{m:i-j-k-}$, as obtained in the first instance under the assumption of independence. The estimates for $\alpha_{m:j}$ and $\alpha_{m:k}$ are obtained in a similar manner. The joint probabilities are re-estimated using this new estimate of $\alpha_{m:i}$ and the current estimates of $\pi_{m:j-k-}$, $\pi_{m:j+k-}$, $\pi_{m:j-k+}$ and $\pi_{m:j+k+}$. This cycle is repeated until convergence.

This method is demonstrated by using the parameter estimates for each country to estimate the numbers of multiple infections for each dataset within that country. These estimates are compared to the observed values and also to the values that would be obtained under the assumption of independence. The method is also applied to datasets where the joint distribution is not specified, but the observed numbers with zero, 1, 2 or 3 infections are given.

RESULTS

Parameter estimates

Initial analyses were conducted separately for each of the study populations listed in Table 1. A χ^2 test was conducted for the null hypothesis that the infections were independent. Data in 107 of the 158 datasets analysed were inconsistent with this hypothesis.

The next stage of the analysis was to determine which of the 2-way or higher order associations were

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

Table 2. Results of combined analyses for country and continent for datasets collected amongst (A) children only and (B) both adults and children reporting mixed infections with the 3 geohelminth species

(The parameter estimates and their 95% confidence intervals are shown, together with the residual deviance and the *P*-value assessing the goodness-of-fit of the final model.)

Area	Datasets	*n*	β_{AT}	β_{AH}	β_{TH}	β_{ATH}	χ^2	D.F.	*P*	
(A)	Benin	1	18512	0·43 (0·26 : 0·60)	−0·44 (−0·55 : −0·33)			11·72	2	0·003
	Kenya	2–4	1352	0·78 (0·54 : 1·02)		0·78 (0·54 : 1·02)		67·33	11	< 0·001
	Madagascar	5–7	798		1·13 (0·80 : 1·46)	1·13 (0·80 : 1·46)		17·28	11	0·10
	Nigeria	8	1060	2·91 (2·38 : 3·43)	2·91 (2·38 : 3·43)	4·96 (3·88 : 6·03)	−5·41 (−6·56 : −4·25)	0·99	1	0·32
	Somalia	9	158			−1·17 (−1·85 : −0·50)		4·79	3	0·19
	Tanzania	10–11	1724	0·68 (0·06 : 1·30)	0·35 (0·06 : 0·64)			1·36	3	0·72
	Africa	1–11	23604	0·51 (0·37 : 0·65)	−0·28 (−0·38 : −0·18)	0·24 (0·08 : 0·39)	0·50 (0·29 : 0·70)	369·39	37	< 0·001
	Indonesia	12–14	158	0·71 (0·11 : 1·31)	0·71 (0·11 : 1·31)	0·71 (0·11 : 1·31)		8·56	11	0·66
	Malaysia	15–24	1645	0·98 (0·74 : 1·23)		0·98 (0·74 : 1·23)		77·85	39	< 0·001
	Sri Lanka	25	100					5·41	4	0·25
	Taiwan	26	937	2·32 (1·97 : 2·67)	2·10 (0·90 : 3·30)	1·18 (0·54 : 1·82)		1·33	1	0·25
	Thailand	27–30	1286	0·53 (0·30 : 0·76)		0·53 (0·30 : 0·76)		39·08	15	< 0·001
	Vietnam	31–38	23545	0·30 (0·19 : 0·40)		0·82 (0·75 : 0·90)		535·42	30	< 0·001
	Asia	12–38	27671	0·57 (0·48 : 0·66)		0·84 (0·76 : 0·91)		884·36	110	< 0·001
	Oceania	39	240					1·49	4	0·83
	N. America	40–43	491	1·71 (1·35 : 2·07)	1·71 (1·35 : 2·07)	1·71 (1·35 : 2·07)		19·86	15	0·18
	S. America	44	520	0·88 (0·49 : 1·26)	n/a	n/a	n/a	0	0	
(B)	Central African Republic	45	3352			0·89 (0·42 : 1·37)		1·79	3	0·62
	Liberia	46	690	1·53 (0·85 : 2·21)	0·58 (0·23 : 0·93)	0·58 (0·23 : 0·93)	−3·44 (−4·80 : −2·08)	0·02	1	0·88
	Madagascar	47	261	3·58 (2·97 : 4·18)	3·58 (2·97 : 4·18)	3·58 (2·97 : 4·18)	−3·58 (−4·18 : −2·97)	3·28	3	0·35
	Nigeria	48–50	1410	1·62 (1·23 : 2·02)	1·42 (1·02 : 1·82)	3·04 (2·50 : 3·57)	−2·59 (−3·22 : −1·96)	97·01	8	< 0·001
	Somalia	51	142	1·57 (0·14 : 3·01)				2·37	3	0·50
	Zaire	52	3056	0·31 (0·19 : 0·43)	0·31 (0·19 : 0·43)			0·19	3	0·98
	Africa	45–52	8911	0·39 (0·30 : 0·47)	0·39 (0·30 : 0·47)	0·75 (0·59 : 0·90)		362·09	30	< 0·001
	China	53–55	1276	0·46 (0·06 : 0·86)	n/a	n/a	n/a	2·71	2	0·26
	India	56	300					91·04	4	< 0·001
	Indonesia	57–59	1387	0·44 (0·27 : 0·60)	0·44 (0·27 : 0·60)	1·04 (0·75 : 1·33)		9·92	10	0·45
	Philippines	60–61	1751	1·19 (0·96 : 1·41)	0·77 (0·48 : 1·06)	0·77 (0·48 : 1·06)		16·30	6	0·01
	Malaysia	62–63	469	1·14 (0·75 : 1·52)				8·71	7	0·27
	Thailand	64	1142	1·51 (1·26 : 1·77)	0·77 (0·19 : 1·34)	1·51 (1·26 : 1·77)		3·02	2	0·22
	Asia	53–64	6325	1·00 (0·89 : 1·11)	0·56 (0·38 : 0·75)	1·00 (0·89 : 1·11)		173·83	37	< 0·001
	Samoa	65	224	1·44 (0·69 : 2·18)		1·44 (0·69 : 2·18)		1·30	3	0·73
	Vanuatu	66	457	1·23 (0·88 : 1·59)		1·23 (0·88 : 1·59)		1·46	3	0·69
	Oceania	65–66	681	1·27 (0·96 : 1·59)		1·27 (0·96 : 1·59)		3·00	7	0·89
	S. America	67	407	1·41 (0·87 : 1·96)	n/a	n/a	n/a	0	0	

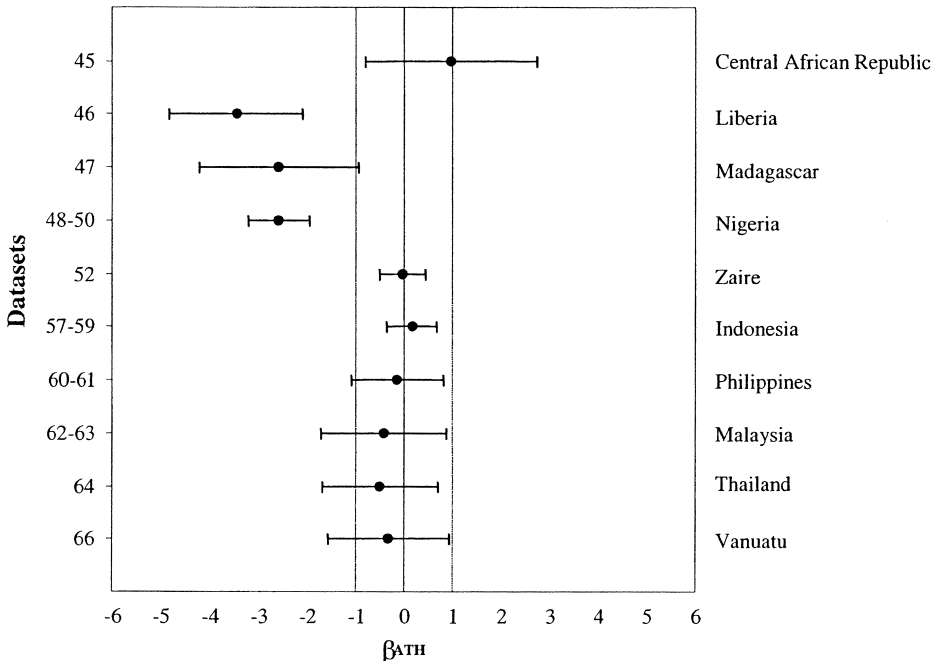

Fig. 1. Estimated values of β_{ATH} and their 95% confidence intervals for the countries in Table 2(B) with estimable 3-way interaction terms.

significant in each population and thus obtain the most parsimonious model describing the data. The results of these analyses are presented in Table 1. Where a significant association was found, the sign of that association is indicated.

For the geohelminth species, the data in 48 of the 65 populations were inconsistent with the null hypothesis of independent infections. In 40 of the populations a significant interaction between *A. lumbricoides* and *T. trichiura* was found; in all but 2 populations this association was positive, indicating that double infections with *A. lumbricoides* and *T. trichiura* were more likely than would be expected under the assumption of independence. Significant pairwise interactions between *A. lumbricoides* and hookworm and between *T. trichiura* and hookworm were also common and generally positive. Evidence for a 3-way association was found in 5 cases.

Of the 73 datasets concerning multiple species malaria infections, significant between-species associations were found in 45. The signs of the associations between malaria species were much less consistent. Of the 22 significant associations found between *P. falciparum* and *P. malariae*, 14 were positive. Associations between *P. falciparum* and *P. vivax* were in general negative (17 out of 26 significant associations). Associations between *P. malariae* and *P. vivax* were less common and in general positive. *P. ovale* and *P. vivax* were present together in just 3 of the datasets and in none of these cases was there a significant interaction between the two species. There was no evidence in any case for a significant 3- or 4-way association.

The next stage of the analysis involved grouping the datasets according to the country in which they were collected and the age of the study population; datasets collected amongst children only were analysed separately from those collected amongst both adults and children. For each of these groups of multiple populations the data were analysed with a view to obtaining the best estimates for the associations in each setting. Countries were grouped according to continent and estimates obtained for each continent in which data were available. Where more than 1 β parameter was significant, equality of the β terms was tested for.

Table 2 shows the results of these grouped analyses for the 3 geohelminth species. In interpreting these results it must be remembered that those individual datasets with the greatest sample sizes will have the biggest influence on the grouped estimates. Those settings in which the final model provides a satisfactory fit to the data are those in which there is no significant heterogeneity amongst the associations in the individual datasets. For example, amongst the 3 datasets collected in Indonesia (12–14, Table 1) there is no evidence for variation in each of the β parameters between the 3 communities and furthermore the hypothesis that each of the associations is equal in value cannot be rejected. Overall, the strongest observation from these tables is that concerning the association between *A. lumbricoides* and *T. trichiura*; although the association is of varying size in different settings, it is in all cases positive. A negative association is observed between *A. lumbricoides* and hookworm in children in Africa, but this association is otherwise positive. With the exception of 1 community in Somalia, the association between *Trichuris* and hookworm was positive for each country and continent.

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

Table 3. Results of combined analyses for country and continent for datasets collected amongst (A) children only and (B) both adults and children reporting mixed infections with the 4 malaria species

(The parameter estimates and their 95% confidence intervals are shown, together with the residual deviance and the *P*-value assessing the goodness-of-fit of the final model.)

Area	Datasets	*n*	β_{FM}	β_{FO}	β_{FV}	β_{MO}	β_{MV}	χ^2	D.F.	*P*
(A)										
Kenya	68–70	485	1·75 (0·94 : 2·57)		n/a	1·03 (0·15 : 1·91)	n/a	18·14	10	0·05
Nigeria	71–75	816	−0·81 (−1·22 : −0·41)			2·78 (1·93 : 3·63)		90·83	15	< 0·001
Sierra Leone	76	809		n/a	2·56 (0·29 : 4·83)	n/a	2·94 (0·61 : 5·26)	0·88	2	0·64
Tanzania	77–78	289			n/a		n/a	3·89	8	0·87
The Gambia	79	1465	0·75 (0·14 : 1·35)		n/a		n/a	2·39	3	0·50
Togo	80	707	0·69 (0·15 : 1·23)	0·69 (0·15 : 1·23)	n/a		n/a	2·25	3	0·52
Uganda	81	2899	1·18 (0·69 : 1·66)	n/a	n/a	n/a	n/a	0	0	
Africa	68–81	7470	0·34 (0·10 : 0·57)	1·52 (0·81 : 2·22)		1·22 (0·77 : 1·68)		193·24	47	< 0·001
China	82	643		n/a	−1·86 (−2·49 : −1·24)	n/a	1·29 (0·80 : 1·79)	100·01	2	< 0·001
India	83–86	1362		n/a	−1·18 (−1·82 : −0·54)	n/a		17·44	12	0·14
Iraq	87	76		n/a		n/a		17·46	4	0·002
Myanmar	88	207		n/a	−1·74 (−2·96 : −0·52)	n/a		19·01	3	< 0·001
Pakistan	89	2891	n/a	n/a		n/a	n/a	2·25	1	0·13
Asia	82–89	5179	−3·30 (−4·30 : −2·30)	n/a	−0·88 (−1·14 : −0·63)	n/a		87·64	24	< 0·001
Oceania	90–92	805	n/a	n/a	−1·95 (−2·81 : −1·09)	n/a	n/a	9·09	2	0·011
(B)										
Gabon	93	2192			n/a		n/a	3·77	4	0·44
Ghana	94	1048	−0·60 (−1·14 : −0·06)		n/a		n/a	0·24	3	0·97
Kenya	95–98	945	2·03 (1·16 : 2·91)		n/a		n/a	34·72	15	0·002
Nigeria	99	159			n/a		n/a	7·05	4	0·13
Senegal	100–101	11004	1·76 (1·58 : 1·94)	1·00 (0·70 : 1·29)	n/a	1·05 (0·86 : 1·24)	n/a	9·30	5	0·098
Sudan	102–103	13552	1·73 (1·61 : 1·86)	1·07 (0·77 : 1·37)	n/a	0·69 (0·42 : 0·95)	n/a	31·05	5	< 0·001
Tanzania	104–106	4203	1·46 (1·23 : 1·69)	n/a	1·95 (1·47 : 2·43)	n/a	1·80 (1·47 : 2·13)	14·26	6	0·027
Africa	93–106	33103	1·61 (1·52 : 1·70)	1·03 (0·82 : 1·23)	1·93 (1·45 : 2·40)	0·93 (0·78 : 1·08)	1·77 (1·44 : 2·09)	181·91	48	< 0·001
Bangladesh	107	1093	n/a	n/a	−5·08 (−5·58 : −4·58)	n/a	n/a	0	0	
China	108–109	2277		n/a		n/a		11·28	8	0·19
India	110–114	4625	−1·16 (−1·68 : −0·64)	n/a	−1·81 (−2·11 : −1·50)	n/a	−1·86 (−3·30 : −0·42)	34·22	14	0·002
Indonesia	115	51						12·28	11	0·34
Israel	116–117	40868	−3·22 (−4·24 : −2·20)	n/a	−1·82 (−2·00 : −1·65)	n/a	−2·27 (−3·69 : −0·85)	1·29	5	0·94
Malaysia	118	268		n/a	0·96 (0·14 : 1·78)	n/a	0·96 (0·14 : 1·78)	1·59	3	0·66
Myanmar	119	151		n/a	−1·29 (−2·12 : −0·33)	n/a		2·82	3	0·42
Pakistan	120–122	7352	n/a	n/a		n/a	n/a	7·45	3	0·06
Sri Lanka	123	11260	−1·13 (−1·51 : −0·75)	n/a	−1·13 (−1·51 : −0·75)	n/a	−1·13 (−1·51 : −0·75)	5·87	3	0·12
Sumatra	124	3266		n/a	0·59 (0·20 : 0·97)	n/a	1·04 (0·56 : 1·51)	1·31	2	0·58

Table 3 (cont.)

Area	Datasets	*n*	β_{FM}	β_{FO}	β_{FV}	β_{MO}	β_{MV}	χ^2	D.F.	*P*
Thailand	125–128	3015	−0·94 (−1·37 : −0·50)		−1·22 (−1·48 : −0·95)	1·47 (0·09 : 2·86)		41·33	24	0·015
Asia	107–128	74226	−0·85 (−1·06 : −0·63)		−1·73 (−1·84 : −1·62)	1·84 (0·51 : 3·17)	−0·38 (−0·60 : −0·16)	806·42	87	< 0·001
Greece	129	87	−1·04 (−1·97 : −0·10)	n/a		n/a		3·17	3	0·37
Macedonia	130	540		n/a		n/a		3·70	4	0·45
Russia	131	1638		n/a		n/a		6·24	4	0·18
Europe	129–131	2265	−1·04 (−1·97 : −0·11)	n/a		n/a		13·00	11	0·29
Papua New Guinea	132	614		n/a	0·99 (0·36 : 1·62)	n/a		3·78	3	0·29
Solomon Islands	133	506		n/a		n/a	3·44 (1·38 : 5·50)	5·47	3	0·14
Vanuatu	134	209	n/a	n/a		n/a	n/a	0·011	1	0·92
Oceania	132–134	1329		n/a		n/a	1·81 (0·97 : 2·66)	24·18	8	0·002
USA	135–136	162		n/a	1·03 (0·14 : 1·92)	n/a		2·65	7	0·92
Mexico	137	2742		n/a	−1·14 (−1·59 : −0·68)	n/a		9·05	3	0·03
N. America	135–137	2904		n/a	−0·73 (−1·09 : −0·27)	n/a		30·90	11	0·001
Brazil	138	86		n/a		n/a	2·92 (1·20 : 4·63)	2·42	3	0·49
Peru	139–140	247	n/a	n/a	n/a	n/a		3·34	2	0·19
S. America	138–140	333		n/a		n/a		16·44	6	0·012

Clearly, even if an estimated parameter is judged not significantly different from zero, it is still possible that a true effect exists. For example, consider the estimated country-specific 3-way interaction terms, β_{ATH}, presented in Table 2(B). Whilst only 3 countries (Liberia, Madagascar and Nigeria) are judged to have significant effects, only 5 of the 10 countries with estimable 3-way interaction terms could reject the null hypothesis that β_{ATH} was equal to -1, whereas 9 of the 10 countries with estimable 3-way interaction terms could reject the null hypothesis that β_{ATH} was equal to 1 (Fig. 1).

Table 3 shows the results of the grouped analyses for the malaria species data. It is much harder in this case to draw consistent conclusions; even for countries belonging to the same continent the associations often have differing signs. For both children and mixed age groups there is a positive association between *P. falciparum* and *P. malariae* in Africa and a negative association between these two species in Asia. For Asian countries, the associations between *P. falciparum* and *P. vivax* were largely negative; however, positive associations were seen between these two species in Tanzania, Papua New Guinea and USA. There were no negative associations involving *P. ovale*; significant associations with both *P. falciparum* and *P. malariae* were always positive. Associations between *P. malariae* and *P. vivax* were quite variable, tending to be negative for Asian countries and positive elsewhere.

Of the datasets containing information on mixed infections with the 2 hookworm species (141–150, Table 1), a positive association was found in 5 cases. Analysing these datasets together ($n = 6820$) resulted in an estimate of 0·49 (95% C.I. 0·31:0·67) for the association between the two species. The residual deviance for this model was 35·37 on 9 degrees of freedom ($P < 0·001$).

Finally, of the 8 datasets classifying individuals with respect to infection with *S. mansoni* and *S. haematobium* a positive association was found in 6 and a negative association was found in just 1 case. A combined analysis of these data resulted in a negative estimate for the between-species association of $-0·14$ (95% C.I. $-0·25:-0·04$), $\chi^2 = 257·63$ on 7 degrees of freedom, $P < 0·001$. This result is due to the influence of the sample size of the single data set providing evidence for a negative association.

Estimation of joint distribution

The numbers of uninfected individuals and single, double and triple infections that would be expected under the hypothesis of independence are compared to the observed values in Fig. 2(A). It is clear that for nearly all geohelminth datasets there are more triple infections than would be predicted under the assumption of independence. There are also more individuals with no infections, and fewer than expected individuals with single or double infections. This is a consequence of the positive between-species associations.

Equivalent plots for the malaria species data are shown in Fig. 3(A); in this case part (iv) corresponds to individuals with 3 or 4 infections. Less consistent patterns are observed here, owing to the range of positive and negative associations between the malaria species. However, there are considerable discrepancies between the observed and expected values, particularly for the numbers with single or double infections.

The best parameter estimates obtained for each country were used in accordance with the described method to estimate the joint distribution of infection and hence the number of individuals harbouring zero, 1, 2 or 3 or more infections. These estimates are compared to the observed values in Figs 2(B) and 3(B). For both the geohelminth and malaria species, it is clear that the expected values using the parameter estimates are much closer to the observed values. Test statistics corresponding to the improvements in fit can be obtained by comparing the sum of the residual deviances under the assumption of independence to the sum of the residual deviances for the best fitting models for each country. This corresponds to an improvement of $\chi^2 = 3097·9$ on 246 degrees of freedom ($P < 0·001$) for the geohelminth datasets and $\chi^2 = 4446·8$ on 244 degrees of freedom ($P < 0·001$) for the malaria species datasets.

Cross-validation

The method for estimation of the joint distribution can be cross-validated on separate datasets which report only the marginal prevalences and the number of individuals with single, double or triple infections. For example, such data are reported from a survey of intestinal helminth infections in schoolchildren in Pemba Island, Tanzania (Albonico *et al*. 1997). Given the marginal prevalences, the parameter estimates for children in Tanzania (see Table 2) were used to estimate the number of children with multiple infections. These estimates were compared to those obtained under the assumption of independence and the results are shown in Table 4(A). The χ^2 statistic comparing observed and expected values is 62·57 under the assumption of independence and 14·95 using the estimated parameters for Tanzania. This results in a χ^2 value for the improvement in fit of 47·62, which is highly significant for a test on 1 degree of freedom ($P < 0·001$). However, since nested models are not being compared, it is not possible to determine the appropriate degrees of freedom for a formal test. Using the parameter estimates means that the number of children with triple infections is estimated more closely than under the assumption of in-

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

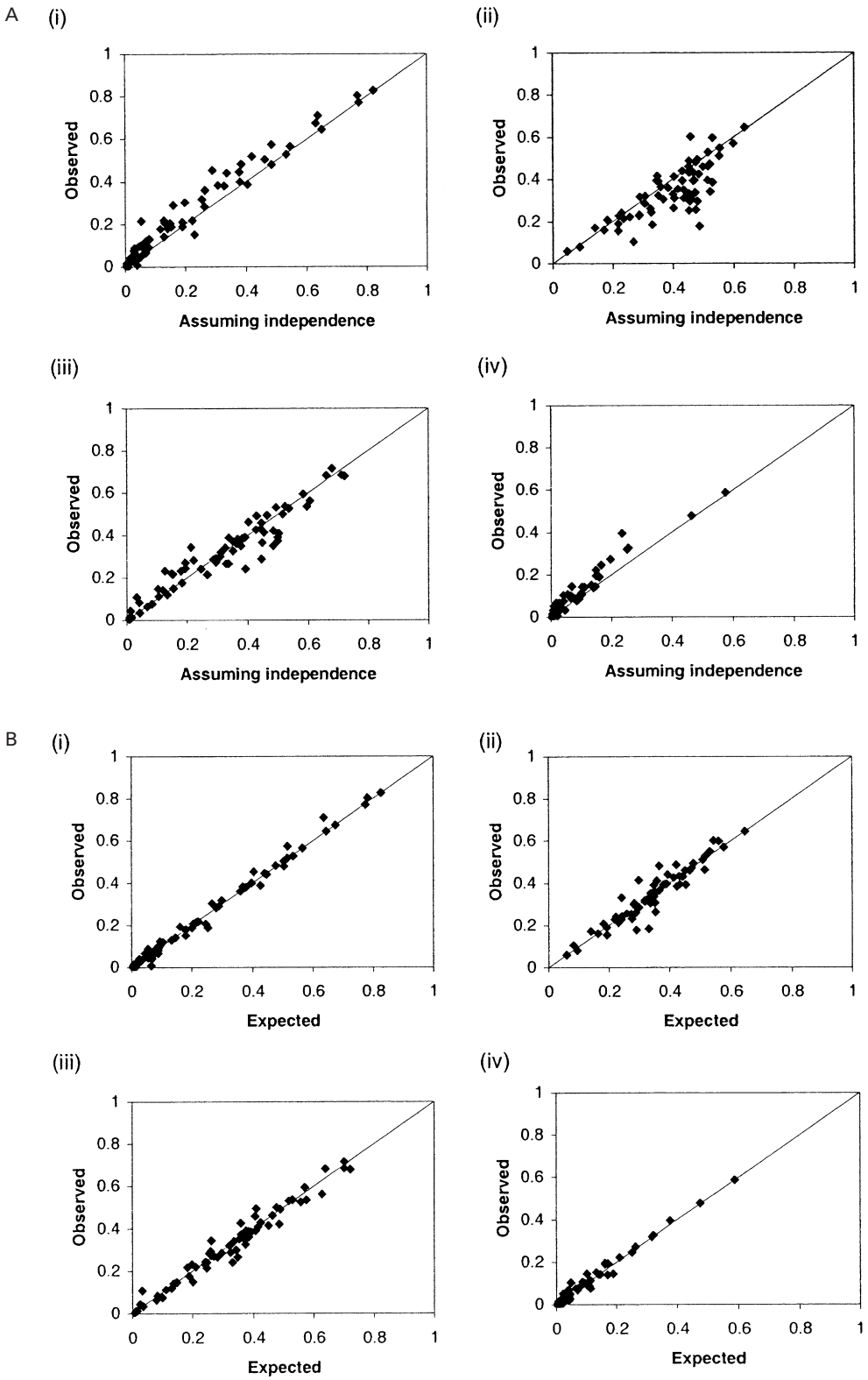

Fig. 2. Observed proportions of individuals harbouring (i) no infections (ii) a single infection, (iii) double infections and (iv) 3 infections with geohelminth species compared to the values that would be expected (A) under the assumption of independence and (B) using the best parameter estimates for that country (see Table 2).

dependence. This is important as it is likely to be those children with multiple infections who are at greatest risk of morbidity and mortality.

As a further example, we consider a dataset reporting the prevalence of intestinal parasites in primary school children in Kampala, Uganda (Kabatereine *et al*. 1997). Prevalences of 10 species of intestinal parasite are reported; however, only the prevalences of *A. lumbricoides*, *T. trichiura*, hookworm and *E. coli* are greater than 1%. For the

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

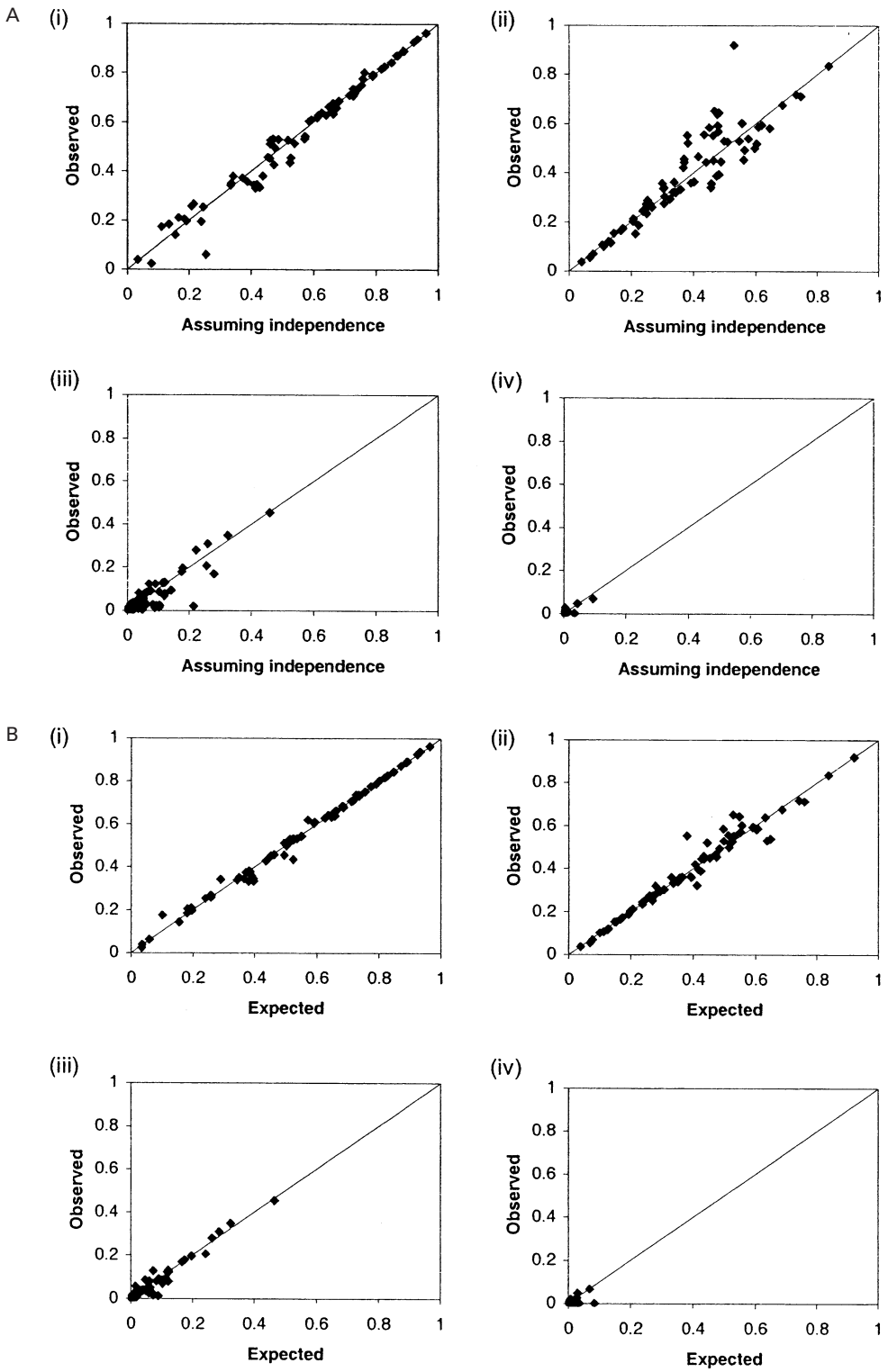

Fig. 3. Observed proportions of individuals harbouring (i) no infections (ii) a single infection, (iii) double infections and (iv) 3 or more infections with malaria species compared to the values that would be expected (A) under the assumption of independence and (B) using the best parameter estimates for that country (see Table 3).

purposes of estimating multiple species infections the remaining species were ignored and the association between *E. coli* and each of the geohelminth species was assumed to be zero. In this case, there are no parameter estimates of the associations available for Uganda; the results of using estimates among children from 2 nearby countries (Tanzania and Kenya) are compared to the results obtained under the assumption of independence and the results are shown in Table 4(B). Again, using either of the sets of estimated associations results in a large improvement in fit, of $\chi^2 = 227{\cdot}8$ and $\chi^2 = 194{\cdot}4$ respectively, of the numbers of children with multiple infections. As above, both these represent significant

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

Table 4. Observed (n_O) and estimated numbers of individuals with multiple infections under the assumption of independence (n_I) and (A) using the parameter estimates (n_P) for children in Tanzania and (B) using the parameter estimates for children in Kenya (n_{P1}) and Tanzania (n_{P2})

No. of infections	n_O	n_I	n_P	
A				
0	1	2·56	4·87	
1	172	105·41	136·05	
2	1009	1162·25	1093·72	
3	2423	2334·78	2370·36	
χ^2 statistic		62·57	14·95	
No. of infections	n_O	n_I	n_{P1}	n_{P2}
B				
0	2535	2621·01	2815·89	2739·76
1	1874	2093·00	1754·57	1888·13
2	740	544·21	638·25	599·41
3	164	54·78	104·24	85·70
χ^2 statistic		314·66	86·86	120·26

improvements ($P < 0{\cdot}001$) compared to 1 degree of freedom, although the appropriate degrees of freedom cannot be determined.

DISCUSSION

Multiple infections have important implications for the practical design of control programmes against parasitic diseases. In localities where parasite species co-occur, control efforts may be best directed against all the species which can be treated by a particular drug rather than each species separately. If targeted chemotherapy is used, a multiple species strategy would be most effective where there is a positive association between infections with different species. Key to the design of such a strategy is a method enabling the quantification of the pattern of multiple infections. Studies of multiple infections are also important for furthering understanding of cross-species immunity and predisposition to infection within hosts.

Data from 47 different countries and over 215 000 individuals have been analysed and a number of between species associations have been investigated. An important point to note with a study such as this is that a large number of statistical tests have been carried out, both within and between populations. This increases the likelihood that one or more of these will produce a significant result merely by chance. Unadjusted P values have been reported in order to allow the reader access to the raw test results independent of a specific correction method; as such results of borderline significance should be interpreted with caution.

The first conclusion from the analysis is that the distribution of multiple species infections is often not independent; data from 107 of the 158 communities analysed were inconsistent with this hypothesis. This implies that simply estimating joint infections by multiplying the overall single-species prevalences together does not give a good estimate of the true numbers of joint infections. We have seen the importance of the between-species associations in terms of estimating the joint distribution of infection.

Intestinal helminth infection adversely affects physical and mental development which is likely to be more serious when multiply infected (Stephenson, 1987). It has been shown that children with multiple species infections have higher egg counts of each species than children with single species infections (Brooker *et al.* 2000) and that individuals with multiple species infections are those at the highest risk of geohelminth-related morbidity (Booth *et al.* 1998*a*). The examination of patterns of multiple infection is therefore crucial in estimating the global health burden of intestinal helminth infections. In general, associations between the helminth species were positive, which implies that more multiple species infections are observed than would be expected under independence. This suggests that there is pre-disposition to multiple infections and that the pre-disposed individuals may be particularly susceptible to disease; practically this implies that efforts should be made to ensure that these individuals are not missed from treatment. Reasons for the positive associations could be due to the transmission routes of the species. *A. lumbricoides* and *T. trichiura* are both transmitted by the faecal–oral route (Booth & Bundy, 1995) which means that social and behavioural factors which lead to infection with one species will increase the probability of

https://doi.org/10.1017/S0031182001007272 Published online by Cambridge University Press

infection with the other species. However, hookworm is transmitted by the percutaneous route and so positive associations between this and either *A. lumbricoides* or *T. trichiura* may be due to increased predisposition to multiple infections in some individuals.

Studies of mixed malaria infections can offer important insights into the mechanisms of immune response within the host, and it is this immune response which will determine the clinical consequences of mixed infections (Mason, McKenzie & Bossert, 1999). It has been suggested that a deficit of mixed infections could be caused by mutual inhibition, where the immune response to one species prevents the invasion of a second (Cohen, 1973). Conversely, it is possible that host heterogeneity could result in an increased frequency of mixed infections, with infections being concentrated in certain susceptible hosts (Molineaux *et al*. 1980). Richie (1988), in a review of interactions between malaria parasites, concluded that the strongest statement that may be made is that suppression or exclusion *may* occur between malarial parasites. McKenzie & Bossert (1999) suggested that the inconsistent findings of previous authors may be due to the different species involved in their studies, with positive associations found by authors analysing *P. falciparum*, *P. malariae*, *P. ovale* data and negative associations generally found when *P. falciparum*, *P. malariae*, *P. vivax* data were analysed. Indeed, we have seen few clear patterns emerging amongst the associations, although it is notable that estimates of the 2-way associations were, in general, positive for African countries and negative for Asian countries.

This considerable geographical heterogeneity in the associations between species is very important in the study of multiple species infections. Questions regarding the causes of this variation still remain to be answered. Heterogeneity amongst the data sets, in terms of both study design and sampling techniques, would be expected to contribute to variation amongst the estimated associations. For example, the crude division of data sets into those concerning children and those concerning all ages will not capture the differences in the distribution of malaria infection in very young, as opposed to older, children. Further, differences in diagnostic techniques may account for differences in the detection of multiple infections between studies. However, it does not seem likely that these factors alone could account for the high observed amounts of variation. In terms of prediction of the joint distribution in a new population, it is thus important to have as comprehensive a range of parameter estimates as possible, in order to estimate the numbers of individuals harbouring multiple infections.

In summary, a general framework has been presented for describing any number of parasite infections, and predicting the joint distribution of infection from marginal prevalences. This formulation allows for interactions between any subset of the parasite species. In principle, although helminth and malarial infections are concentrated on here, the model can be applied to all types of infections including bacterial and protozoan infections. Here, we have concentrated upon infection status defined by presence or absence of infection, but the model could also be used to investigate multiple occurrences of infections above a certain defined level. In particular, it would be of interest to determine if individuals with an intense infection of one species are more likely to experience an intense infection with another species. Needham *et al*. (1998) demonstrated that high intensity *A. lumbricoides* infections were significantly more likely to occur with high intensity *T. trichiura* infections than would be expected by chance. Identification of those individuals with multiple intense infections is important not just for the individuals concerned but also in terms of reducing transmission in the environment. As such, it will be of value to quantify the associations between multiple intense infections in different geographical settings.

S.C.H. thanks the Medical Research Council for Studentship Funding. C.A.D. thanks the Wellcome Trust for research funding. M.S.C. held a Wellcome Trust Training Fellowship in Biomathematics at the time of this study. We thank Simon Brooker for access to his collection of references on geohelminth infections in African countries.

REFERENCES

ADEYEBA, O. & DIPEOLU, O. (1984). A survey of gastrointestinal parasites in a local government area of south-west Nigeria. *International Journal of Zoonoses* **11**, 105–110.

AHMED, E., DAFFALLA, A., CHRISTENSEN, N. & MADSEN, H. (1996). Patterns of infection and transmission of human schistosomiasis mansoni and schistosomiasis haemotobium in White Nile Province, Sudan. *Annals of Tropical Medicine and Parasitology* **90**, 173–180.

ALBONICO, M., CHWAYA, H., MONTRESOR, A., STOLFZFUS, R., TIELSCH, J., ALAWI, K. & SAVIOLI, L. (1997). Parasitic Infections in Pemba Island Schoolchildren. *East African Medical Journal* **74**, 294–298.

ALIFRANGIS, M., LEMNCE, M., MOON, R., THEISEN, M., BYGBJERG, I., RIDLEY, R. & JAKOBSEN, P. (1999). IgG reactivities against recombinant rhoptry-associated protein-1 (rRAP-1) are associated with mixed *Plasmodium* infections and protection against disease in Tanzanian children. *Parasitology* **119**, 337–342.

ALONZO, E., ALONZO, V., FORINO, D. & IOLI, A. (1993). Parasitological research in Zinvie (Benin) from 1982 to 1991. *Medecine Tropicale* **53**, 331–335.

BANCHONGAKSORN, T., YOMOKGUL, P., PANYIM, S., ROONEY, W. & VICKERS, P. (1996). A field trial of the *Para*Sight–Ftest for the diagnosis of *Plasmodium falciparum* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 244–245.

BARBIER, D., MOREAU, J.-P. & RADANIELINA, R. (1975). Enquete épidémiologique auprés de compagnies des forces armées du sud-est de Madagascar. *Archives de l'institute Pasteur de Madagascar* **44**, 115–126.

BEDIER, E., LARUENCY & BAURY (1924). Index endemique du paludisme a Niamey a la fin de la saison des pluies. *Bulletin de la Société de Pathologie Exotique* **XVII**, 327.

BOOTH, M. & BUNDY, D. (1992). Comparative prevalences of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections and the prospects for combined control. *Parasitology* **105**, 151–157.

BOOTH, M. & BUNDY, D. (1995). Estimating the number of multiple-species geohelminth infections in human communities. *Parasitology* **111**, 645–653.

BOOTH, M., BUNDY, D., ALBONICO, M., CHWAYA, H. & ALAWI, K. (1998*a*). Associations among multiple geohelminth species infections in schoolchildren from Pemba Island. *Parasitology* **116**, 85–93.

BOOTH, M., LI, Y. & TANNER, M. (1996). Helminth infections, morbidity indicators and schistosomiasis treatment history in three villages, Dongting region, PR China. *Tropical Medicine and International Health* **1**, 464–474.

BOOTH, M., MAYOMBANA, C. & KILIMA, P. (1998*b*). The population biology and epidemiology of schistosome and geohelminth infections among schoolchildren in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 491–495.

BOREL, M. & LEVANAN (1927). Le paludisme à Saigon. *Bulletin de la Société de Pathologie Exotique*. **XX**, 94.

BROOKER, S., BOOTH, M. & GUYATT, H. (1999*a*). Comparisons of schistosome and geohelminth infection prevalences in school-aged children from selected areas of Africa: implications for rapid assessment and combined control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 125–126.

BROOKER, S., MIGUEL, E., MOULIN, S., LUOBA, A., BUNDY, D. & KREMER, M. (2000). Epidemiology of single and multiple species of helminth infections among school children in Busia District, Kenya. *East African Medical Journal* **77**, 157–161.

BROOKER, S., PESHU, N., WARN, P., MOSOBO, M., GUYATT, H., MARSH, K. & SNOW, R. (1999*b*). The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan Coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 240–246.

BROWN, R., WILKS, N. & ALLEN, D. (1970). Health survey of primary schoolchildren in Uganda: incidence of anaemia, splenomegaly, hookworm and malaria. *East African Medical Journal* **47**, 302–318.

BUNDY, D., CHANDIWANA, S., HOMEIDA, M., YOON, S. & MOTT, K. (1991). The epidemiological implications of a multiple-infection approach to the control of human helminth infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 274–276.

BUXTON, P. (1927). *Researches in Polynesia and Melanesia I*. London School of Hygiene and Tropical Medicine, London.

CABRERA, B., CABALLERO, B., RAMPAL, L. & DE LEON, W. (1989*a*). Philippines. In *Ascariasis and its Prevention and Control*, pp. 169–183. Taylor and Francis, London.

CABRERA, B., LORENZO, M., ABRANTES, W., GO, T. & ORTIZ, C. (1989*b*). An attempt to eradicate *Ascaris* and Hookworm infections in an island in Sorsogon, Philippines for a three-year period. *Collected Papers on the Control of Soil-Transmitted Helminthiases*, Vol. IV, 105–120.

CAMPBELL, G., COLLINS, F., BRANDLING-BENNETT, A., SCHWARTZ, I. & ROBERTS, J. (1987). Age-specific prevalence of antibody to a synthetic peptide of the circumsporozoite protein of *Plasmodium falciparum* in children from three villages in Kenya. *American Journal of Tropcial Medicine and Hygiene* **39**, 220–224.

CARDAMATIS, J. (1911). Die Sanierung von Neu-Anchialos. *Archiv für Schiffs und Tropenhygiene Beihefte* **XV**, 441.

CARME, B. (1984). A study of hookworm infection among children in Brazzaville (People's Republic of the Congo). *Bulletin de la Société de Pathologie Exotique* **77**, 320–327.

CHAN, M.-S. (1997). The global burden of intestinal nematode infections – fifty years on. *Parasitology Today* **13**, 438–443.

CHAN, M.-S., MEDLEY, G., JAMISON, D. & BUNDY, D. (1994). The evaluation of potential global morbidity attributable to intestinal nematode infections. *Parasitology* **109**, 373–387.

CHANDIWANA, S., BRADLEY, M. & CHOMBO, F. (1989). Hookworm and roundworm infections in farm-worker communities in the large-scale agricultural sector in Zimbabwe. *Journal of Tropical Medicine and Hygiene* **92**, 338–344.

CHEN, E.-R. (1964). A study of soil-transmitted helminthic infections and health in pre-schoolchildren of rural villages in South Taiwan. *Journal of the Formosan Medical Association* **63**, 517–530.

CHRISTOPHERS, S. & SHORTT, H. (1921). Malaria in Mesopotamia. *Indian Journal of Medical Research* **VIII**, 508.

COHEN, J. (1973). Heterologous immunity in human malaria. *The Quarterly Review of Biology* **48**, 467–489.

COLLINS, W., CATTANI, J., LOURIE, J., TAUFA, T., ANDERSON, W., SKINNER, J., STANFILL, P. & HUONG, A. (1988). Antibody responses to malarial antigens in the Wopkaimin population of the star mountains, Papua New Guinea. *American Journal of Tropical Medicine and Hygiene* **39**, 241–245.

CREASEY, A., WESSELS, J. & CLARKE, V. D. V. (1982). Re-evaluation of Criteria for Making a Diagnosis of Bilharzia. *The Central African Journal of Medicine* **28**, 265–272.

DAUBNEY, R. & CARMAN, J. (1928). Helminthic infestations of natives in the Kenya Highlands. *Parasitology* **20**, 185–206.

DAZO, B. (1989). Western Pacific region. In *Ascariasis and its Prevention and Control*, pp. 185–205. Taylor and Francis, London.

DE SILVA, D. & JAYATILLEKA, S. (1981). The prevalence and severity of soil transmitted helminths in an urban slum community in Colombo. *Ceylon Medical Journal* **26**, 160–164.

DELORON, P., CAMPBELL, G., BRANDLING-BENNETT, D., ROBERTS, J., SCHWARTZ, I., ODERA, J., LAL, A., OSANGA,

C., CRUZ, V. D. L. & MCCUTCHAN, T. (1989). Antibodies to *Plasmodium falciparum* ring-infected erythrocyte surface antigen and *P. falciparum* and *P. malariae* circumsporozoite proteins: seasonal prevalence in Kenyan villages. *American Journal of Tropical Medicine and Hygiene* **41**, 395–399.

DHANACHAND, C. & ANAND, L. (1997). A survey on prevalence of intestinal nematode infection in two urban populations of Imphal, Manipur, India. *Journal of Parasitology and Applied Animal Biology* **6**, 103–107.

DOROLLE, P. (1927). Le paludisme a Haigang (Tonkin). *Bulletin de la Société de Pathologie Exotique* **XX**, 895.

EVERITT, B. (1997). *The Analysis of Contingency Tables*. Chapman and Hall, London.

FERREIRA, C., FERREIRA, M. & NOGUEIRA, M. (1994). The prevalence of infection by intestinal parasites in an urban slum in Sao-Paulo, Brazil. *Journal of Tropical Medicine and Hygiene* **97**, 121–127.

FIENBERG, S. (1970). The analysis of multidimensional contingency tables. *Ecology* **51**, 419–433.

FULMER, H. & HUEMPFNER, H. (1965). Intestinal helminths in Eastern Kentucky: a survey in three rural counties. *American Journal of Tropical Medicine and Hygiene* **14**, 269–275.

GBARY, A., GUIGUEMDÉ, T. & OUEDRAOGO, J. (1988). Emergence of chloroquine resistant malaria in West Africa: the case of Sokode in Togo. *Tropical Medicine and Parasitology* **39**, 142–144.

GORDON, D., DAVIS, D., LEE, M., LAMBROS, C., HARRISON, B., SAMUEL, R., CAMPBELL, G. M. J., SELVARAJAN, K. & LEWIS, G. (1991). Significance of circumsporozoite-specific antibody in the natural transmission of *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* in an aboriginal (Orang Asli) population of central peninsular Malaysia. *American Journal of Tropical Medicine and Hygiene* **45**, 49–56.

GRIFFITTS, T. (1925). Impounded waters and malaria. *Southern Medical Journal* **XIX**, 367.

HASWELL-ELKINS, M., ELKINS, D. & ANDERSON, R. (1987). Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius* and *Trichuris* in a South Indian fishing community. *Parasitology* **95**, 323–337.

HELLGREN, U., ERICSSON, O., KIHAMIA, C. & ROMBO, L. (1994). Malaria parasites and chloroquine concentrations in Tanzanian schoolchildren. *Tropical Medicine and Parasitology* **45**, 293–297.

HIGGINS, D., JENKINS, D., KURNIAWAN, L., PURNOMO, HARUN, S. & JU-WONO, S. (1984). Human intestinal parasitism in three areas of Indonesia: a survey. *Annals of Tropical Medicine and Parasitology* **78**, 637–648.

HOLLAND, C., ASAOLU, S., CROMPTON, D., STODDART, R., MACDONALD, R. & TORIMIRO, S. (1989). The epidemiology of *Ascaris lumbricoides* and other soil-transmitted helminths in primary school children from Ile-Ife, Nigeria. *Parasitology* **99**, 275–285.

HOTEZ, P., ZHENG, F., LONG-QI, X., MING-GANG, C., SHU-HUA, X., SHU-XIAN, L., BLAIR, D., MCMANUS, D. & DAVIS, G. (1997). Emerging and reemerging helminthiases and the public health of China. *Emerging Infectious Diseases* **3**, 303–310.

HSIEH, H., STOLL, N., REBER, E., CHEN, E., KANG, B. & KUO, M. (1972). Distribution of *Necator americanus* and *Ancylostoma duodenale* in Liberia. *Bulletin of the World Health Organization* **47**, 317–324.

ILARDI, I., KHALIF, B., BIANCHINI, C., SANGUIGNI, S., ACETI, A., PAPARO, B., MARANGI, M., LEONE, F., DI PIRAMO, D., CALI, S., PENNICA, A. & TESSITORE, S. (1985). An epidemiological survey on the parasitological diseases by means of a direct and immunodiagnostical method in a boys' boarding-school in Somalia. *Giornale di Malattie Infettive e Parassitaire* **37**, 248–250.

ILARDI, I., PETRACCA, C., CAPPELLI, A., PELLE, P. & FARAH, A. (1980). Indagine epidemiologica con metodo diretto e immunodiagnostico delle malattie parassitaire nei residenti di un villaggio Somalo situato in zona irrigata. *Parasitologica* **XXII**, 141–148.

ISMID, I., RASAD, R. & RUKOMO, B. (1981). Prevalence and treatment of intestinal helminthic infections among children in orphanages in Jakarta, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **12**, 371–375.

JANCLOES, M.-F. & CORNET, P. (1980). Epidemiological control of intestinal helminths in a rural area of Zaire. *Revue d'Epidémiologie et de Santé Publique* **28**, 89–103.

JEMANEH, L. (1998). Comparative prevalences of some common intestinal helminth infections in different altitudinal regions in Ethiopia. *Ethiopian Medical Journal* **36**, 1–8.

KABATEREINE, N., KEMIJUMBI, J., KAZIBWE, F. & ONAPA, A. (1997). Human intestinal parasites in primary school children in Kampala, Uganda. *East African Medical Journal* **74**, 311–314.

KAN, S. (1984). Soil-transmitted helminthiasis among primary school children in Selangor, Malaysia. *Medical Journal of Malaysia* **39**, 143–147.

KAN, S. (1986). Prevalence of soil-transmitted helminthiases among urban and rural school children in Malaysia. *Collected Papers on the Control of Soil Transmitted Helminthiases*, Vol. III, 32–36.

KAN, S. (1993). Environmental, socioeconomic and cultural-behavioural factors affecting endemicity of soil-transmitted helminthiases and nutritional status of urban slum dwellers. *Collected Papers on the Control of Soil-Transmitted Helminthiases*, Vol. V, 44–63.

KIGHTLINGER, L., SEED, J. & KIGHTLINGER, M. (1995). The epidemiology of *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm in Children in the Ranomafana rainforest, Madagascar. *Journal of Parasitology* **81**, 159–169.

KINOTI, G. (1971). The prevalence of helminth infections in the Kisumu area of Kenya. *East African Medical Journal* **48**, 490–495.

KNOWLES, R. & WHITE, R. (1930). Studies in the parasitology of malaria. *Indian Medical Research Memoirs* **18**, 1–436.

LANDGRAF, B., KOLLARITSCH, H., WEIDERMANN, G. & WERNSDORFER, W. (1994). Parasite density of *Plasmodium falciparum* malaria in Ghanian schoolchildren: evidence for influence of sex hormones? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 73–74.

LEGER, M., PINAUD, J. & BEDIER, E. (1923). Le paludisme a Zinder: index hematologique. *Bulletin de la Société de Pathologie Exotique* **XVI**, 137.

LOUM, C. (1974). A preliminary report on epidemiology and control of schistosomiasis at Kisangra. *East African Journal of Medical Research* **1**, 125–131.

LWAMBO, N., SIZA, J., BROOKER, S., BUNDY, D. & GUYATT, H. (1999). Patterns of concurrent hookworm infection and schistosomiasis in schoolchildren in Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 497–502.

MAFIANA, C., SODIPE, M. & KOLEOSO, B. (1998). Soil transmitted helminth parasites of humans in a city in southwestern Nigeria. *Helminthologica* **35**, 203–208.

MAITLAND, K., WILLIAMS, T., BENNETT, S., NEWBOLD, C., PETO, T., VIJI, J., TIMOTHY, R., CLEGG, J., WEATHERALL, D. & BOWDEN, D. (1996). The interaction between *Plasmodium falciparum* and *P. vivax* in children on Espiritu Santo Island, Vanuatu. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 614–620.

MARCH'HADOUR, P., CARRE, J. & DODIN, A. (1960). Enquete bacteriologique et parasitologique a l'ile Sainte-Marie. *Archives de l'institute Pasteur de Madagascar* **28**, 145–146.

MASON, D., McKENZIE, F. & BOSSERT, W. (1999). The blood-stage dynamics of mixed *Plasmodium malariae* – *Plasmodium falciparum* infections. *Journal of Theoretical Biology* **198**, 549–566.

MASTERMAN, E. (1913). Notes on some tropical diseases of Palestine. *Journal of Hygiene* **XIII**, 49.

MAY, J., MOCKENHAUPT, F., ADEMOWO, O., FALUSI, A., OLUMESE, P., BIENZLE, U. & MEYER, C. (1999). High rate of mixed and subpatent malarial infections in southwest Nigeria. *American Journal of Tropical Medicine and Hygiene* **61**, 339–343.

McKENZIE, F. & BOSSERT, W. (1997). Mixed species *Plasmodium* infections of humans. *Journal of Parasitology* **83**, 593–600.

McKENZIE, F. & BOSSERT, W. (1999). Multispecies *Plasmodium* infections of humans. *Journal of Parasitology* **85**, 12–18.

MEAKINS, R., HARLAND, P. & CARSWELL, F. (1981). A preliminary survey of malnutrition and helminthiasis among school-children in one mountain and one lowland ujamaa village in Northern Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 731–735.

MEUNIER, D., GEORGES, M. & GEORGES, A. (1984). Intestinal parasitosis of adults in a town of Central African Republic. *Bulletin de la Société de Pathologie Exotique* **77**, 333–343.

MIZUSHIMA, Y., KATO, H., OHMAE, H., TANAKA, T., BOBOGARE, A. & ISHII, A. (1994). Prevalence of malaria and its relationship to anemia, blood glucose levels, and serum somatomedin c (IGF-1) levels in the Solomon Islands. *Acta Tropica* **58**, 207–220.

MOLINEAUX, L., STOREY, J., COHEN, J. & THOMAS, A. (1980). A longitudinal study of human malaria in the West African Savanna in the absence of control measures: relationships between different *Plasmodium* species, in particular *P. falciparum* and *P. malariae*. *American Journal of Tropical Medicine and Hygiene* **29**, 725–737.

NEEDHAM, C., KIM, H., CONG, L., MICHAEL, E., DRAKE, L., HALL, A. & BUNDY, D. (1998). Epidemiology of soil-transmitted nematode infections in Ha Nam Province, Vietnam. *Tropical Medicine and International Health* **3**, 904–912.

NHONOLI, A., KIHAMA, F. & RAMJI, B. (1974). Infection rates of Tanzanian rural communities by the common parasites and some of their haematological effects. *East African Medical Journal* **51**, 829–836.

NOSTEN, F., MAELANKIRRI, L., DECLUDT, B. & WHITE, N. (1991). Malaria during pregnancy in an area of unstable endemicity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 424–429.

ONADEKO, M. & LAPIDO, O. (1989). Intestinal parasitic infestation in rural communities: a focus for primary health care in Nigeria. *African Journal of Medicine and Medical Sciences* **18**, 289–294.

PERRY, E. (1911). Malaria in the Jeypore hill tract and adjoining coast land. *Paludism* **3**, 35.

PETNEY, T. & ANDREWS, R. (1998). Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *International Journal for Parasitology* **28**, 377–393.

PURNOMO, SOLIHIN, A., GOMEZ-SALADIN & BANGS, M. (1999). Rare quadruple malaria infection in Irian Jaya Indonesia. *Journal of Parasitology* **85**, 574–579.

RICHIE, T. (1988). Interactions between malaria parasites infecting the same vertebrate host. *Parasitology* **96**, 607–639.

ROSENBERG, R. & MAHESWARY, N. (1982). Forest malaria in Bangladesh I. Parasitology. *American Journal of Tropical Medicine and Hygiene* **31**, 175–182.

SALDIVA, S., SILVEIRA, A., PHILIPPI, S., TORRES, D., MANGINI, A., SOUZA DIAS, R. D., DA SILVA, R., BURATINI, M. & MASSAD, E. (1999). *Ascaris-Trichuris* association and malnutrition in Brazilian children. *Paediatric and Perinatal Epidemiology* **13**, 89–98.

SALEM, G., VAN DE VELDEN, L., LALOÉ, F., MAIRE, B., PONTON, A., TRAISSAC, P. & PROST, A. (1994). Intestinal parasitic infection and environment in Sahelo-Sudanese towns: the case of Pikine (Senegal). *Revue d'Epidémiologie et de Santé Publique* **42**, 322–333.

SCHUFFNER, W. (1938). Two subjects relating to the epidemiology of malaria. *Journal of the Malaria Institute of India* **1**, 221–256.

SINNIAH, B., SINNIAH, D., SINGH, M. & POON, G. (1978). Prevalence of parasitic infections in Malaysian oil palm estate workers. *Southeast Asian Journal of Tropical Medicine and Public Health* **9**, 272–276.

SNOUNOU, G., VIRIYAKOSOL, S., JARRA, W., THAITHONG, S. & BROWN, K. (1993). Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Molecular and Biochemical Parasitology* **58**, 283–292.

STEPHENSON, L. (1987). *Impact of Helminth Infections on Human Nutrition : Schistosomes and Soil Transmitted Helminths*. Taylor and Francis, New York.

STRICKLAND, G., FOX, E. & HADI, H. (1988). Malaria and splenomegaly in th.ee Punjab. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 667–670.

STRICKLAND, G., ZAFAR-LATIF, A., FOX, E., KHALIQ, A. & CHOWDHRY, M. (1987). Endemic malaria in four villages of the Pakistani province of Punjab. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 36–41.

STÜRCHLER, D., STAHEL, E., SALADIN, K. & SALADIN, B. (1980). Intestinal parasites in eight Liberian settlements: Prevalences and community anthelmintic chemoterapy. *Tropenmedizin und Parasitologie* **31**, 87–93.

STURROCK, R. (1966*a*). Hookworm studies in Tanganyika (Tanzania): investigations at Hombolo in the Dodoma region. *East African Medical Journal* **43**, 315–322.

STURROCK, R. (1966*b*). Hookworm studies in Uganda: investigations at Teboke in Lango District. *East African Medical Journal* **43**, 430–438.

SULZER, A., CANTELLA, R., COLICHON, A., GLEASON, N. & WALLS, K. (1975). A focus of hyperendemic *Plasmodium malariae – P. vivax* with no *P. falciparum* in a primitive population in the Peruvian Amazon jungle. *Bulletin of the World Health Organization* **52**, 273–278.

THOMSON, M., D'ALESSANDRO, U., BENNETT, S., CONNOR, S., LANGEROCK, P., JAWARA, M., TODD, J. & GREENWOOD, B. (1994). Malaria prevalence is inversely related to vector density in The Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 638–643.

TOAN, N. (1993). Prevalence of soil-transmitted helminthiasis among children aged, 4–15 in Vietnam. *Collected Papers on the Control of Soil-Transmitted Helminthiases*, Vol. V, 111–120.

TRAPE, J.-F., LEFEBVRE-ZANTE, E., LEGROS, F., NDIAYE, G., BOUGANALI, H., DRUILHE, P. & SALEM, G. (1992). Vector density gradients and the epidemiology of urban malaria in Dakar, Senegal. *American Journal of Tropical Medicine and Hygiene* **47**, 181–189.

TRAPE, J.-F., ROGIER, C., KONATE, L., DIAGNE, N., BOUGANALI, H., CANQUE, B., LEGROS, F., BADJI, A., NDIAYE, G., NDIAYE, P., BRAHIMI, K., FAYE, O., DRUILHE, P. & DA SILVA, L. (1994). The Dielmo project: a longitudinal study of natural malaria infection and the mechansims of protective immunity in a community living in a holoendemic area of Senegal. *American Journal of the Tropical Medicine and Hygiene* **51**, 123–137.

TREADGOLD, C. (1918). Malaria in Macedonia with especial reference to the use of prophylactic quinine. *Journal of the Royal Army Medical Corps* **XXX**, 571.

UDONSI, J. (1984). Studies on the co-occurrence of two species of human hookworm in a riverine community in Nigeria. *Tropenmedizin und Parasitologie* **35**, 37–40.

UDONSI, J., BEHNKE, J. & GILBERT, F. (1996). Analysis of the prevalence of infection and associations between human gastrointestinal nematodes among different age classes living in the urban and suburban communities of Port Harcourt, Nigeria. *Journal of Helminthology* **70**, 75–84.

UPATHAM, E., VIYANANT, V., BROCKELMAN, W., KURATHONG, S. P. L. & CHIDAPHOL, U. (1989). Prevalence, incidence, intensity and associated morbidity of intestinal helminths in South Thailand. *International Journal for Parasitology* **19**, 217–228.

WILDING, E., WINKLER, S., KREMSNER, P., BRANDTS, C., JENNE, L. & WERNSDORFER, W. (1995). Malaria epidemiology in the province of Moyen Ogoov, Gabon. *Tropical Medicine and Parasitology* **46**, 77–82.

WILSON, D. (1936). Rural hyper-endemic malaria in Tanganyika territory. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **29**, 583–618.

YAKIMOFF, W. (1923). Sur la question du paludisme au Turkestan russe. *Bulletin de la Société de Pathologie Exotique* **XVI**, 243.

YOKOGAWA, M., VAJRASTHIRA, S., VIKAKUL, C., ANANTAPHRUTI, M., YINGYOURD, P., TSUJI, M. & KOJIMA, S. (1983). Control of soil-transmitted helminthiasis and its impact on the nutritional status. *Collected Papers on the Control of Soil Transmitted Helminthiases*, Vol. II, 295–309.

ZHOU, M., LIU, Q., WONGSRICHANALAI, SUWONKERD, W., PANART, K., PENSIRI, A., KIMURA, M., MATSUOKA, H., FERREIRA, M., ISOMURA, S. & KAWAMOTO, F. (1998). High prevalence of *Plasmodium malariae* and *Plasmodium ovale* in malaria patients along the Thai-Myanmar border, as revealed by acridine orange staining and PCR- based diagnoses. *Tropical Medicine and International Health* **3**, 304–312.