Childhood *Cryptosporidium* infection among aboriginal communities in Peninsular Malaysia

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Cryptosporidium is a coccidian parasite that is prevalent worldwide, some species of which cause morbidity in both immunocompromised and immunocompetent individuals. The prevalence and predictors of *Cryptosporidium* infection, and its effect on nutritional status, have recently been explored among 276 children (141 boys and 135 girls, aged 2–15 years) in aboriginal (Orang Asli) villages in the Malaysian state of Selangor. Faecal smears were examined by the modified Ziehl–Neelsen staining technique while socio–economic data were collected using a standardized questionnaire. Nutritional status was assessed by anthropometric measurements. *Cryptosporidium* infection, which was detected in 7.2% of the aboriginal children, was found to be significantly associated with low birthweight (⩽2.5 kg), being part of a large household (with more than seven members) and prolonged breast feeding (>2 years). The output of a binary logistic regression confirmed that large household size was a significant predictor of *Cryptosporidium* infection (giving an odds ratio of 2.15, with a 95% confidence interval of 1.25–5.02). *Cryptosporidium* infection is clearly a public-health problem among the aboriginal children of Selangor, with person-to-person the most likely mode of transmission.

The intracellular coccidian parasites in the genus *Cryptosporidium* have a world-wide distribution (Fayer, 2004). In humans, infection with some *Cryptosporidium* species can cause symptomatic disease (cryptosporidiosis) — usually acute but self-limiting diarrhoea in the immunocompetent and life-threatening diarrhoea in the immunocompromised, particularly patients receiving immunosuppressive drugs and AIDS cases (Flanigan *et al.*, 1992). Cryptosporidiosis has been recorded as the cause of death of an AIDS patient (Current *et al.*, 1983) and clearly has general public-health significance since it can contribute to malnutrition among children in resource-poor areas of the tropics (Molbak *et al.*, 1994*a*; Agnew *et al.*, 1998; Checkley *et al.*, 1998).

In Malaysia, *Cryptosporidium* is gaining in importance because of the increasing numbers of HIV-positive humans in the country. Even 5 years ago, approximately 0.3% of the Malaysian population was estimated to be HIV-positive, with a mean of 17 new cases of HIV/AIDS occurring each day (Anon., 2005). The prevalence of cryptosporidiosis among HIV-positive patients in the country has been found to range from 3% to 23% (Kamel *et al.*, 1994; Lim *et al.*, 2005) and *Cryptosporidium* infection has also been detected in 0.9%–11.4% of children attending hospitals in Malaysia (Lim *et al.*, 2008) and in 10.6% of the children surveyed in a community-based study (Lai, 1992). The predictors of *Cryptosporidium* infection

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among the aboriginal children of Malaysia and the effect of such infection on the health of such children are, however, still unclear. The main aims of the present study were to determine the prevalence and predictors of *Cryptosporidium* infection in aboriginal children from the Malaysian state of Selangor and to assess the effect of such infection, if any, on the children's nutritional status.

SUBJECTS AND METHODS

Study Area and Subjects

A community-based cross-sectional study was carried out, between the January and April of 2004, among the children in eight aboriginal (Orang Asli) villages in the districts of Gombak, Hulu Selangor and Kuala Kubu Baru, in Selangor, Malaysia (see Figure). Most of the adult residents of these villages work as labourers, farmers or rubber tappers or do other 'odd' jobs, such as selling forest products. Most of the houses have electricity only during the night, and most households have a piped supply of drinking water but collect water for their animals, bathing and washing clothes and utensils from nearby rivers. None of the study villages has adequate sanitation. A single health clinic serves all eight villages and this is equipped with an ambulance so that any critical cases can be transferred to the nearest hospital, which is about 50 km away.

Although attempts were made, in each study village, to enroll every child aged <16 years, a child was only enrolled with his or her agreement (if the child was aged >5 years) and the written, informed consent of his or her parents/guardians.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethics Committee of the Faculty of Medicine at the Universiti Kebangsaan Malaysia, in Kuala Lumpur.

Parasitology

Over many visits to the villages, a single faecal sample was collected from each subject. Smears of these samples were prepared and stained with modified Ziehl–Neelsen stain (Henriksen and Pohlenz, 1981) for the detection of *Cryptosporidium* oocysts. Briefly, smears were air dried, fixed in methanol, stained with carbol fuchsin for 10 min, decolourized with acid alcohol for 10–15 s, and then stained with Malachite Green for 3 min. Stained smears were examined, for the presence of oocysts and microsporidial spores, under a light microscope at $\times 1000$.

Standard Questionnaire

Demographic and socio–economic data on each subject (see Results) were collected by trained assistants, in interviews with the subjects and their parents/guardians in their villages, using a standardized questionnaire that had been constructed in English and then translated into Malay, the local language.

Assessment of Nutritional Status

Each subject was given a physical examination during which his or her weight and height were recorded. The children were weighed, clothed but without their shoes, using calibrated Seca 709 electronic scales (Seca, Hamburg, Germany). Height was measured by standing the child against a vertical wall, holding a clipboard so that it touched the wall and lay horizontally across the top of the child's head, and marking the wall where the clipboard touched it. Weight-for-age, height-for-age and weight-for-height z-scores were then calculated as indicators for underweight, stunting (chronic malnutrition) and wasting (acute malnutrition), respectively. The z-scores were calculated, using reference data from the United States National Centre for Health Statistics (NCHS) and the World Health Organization, in the Epi-Info for Windows 2002 software package (Centers for Disease

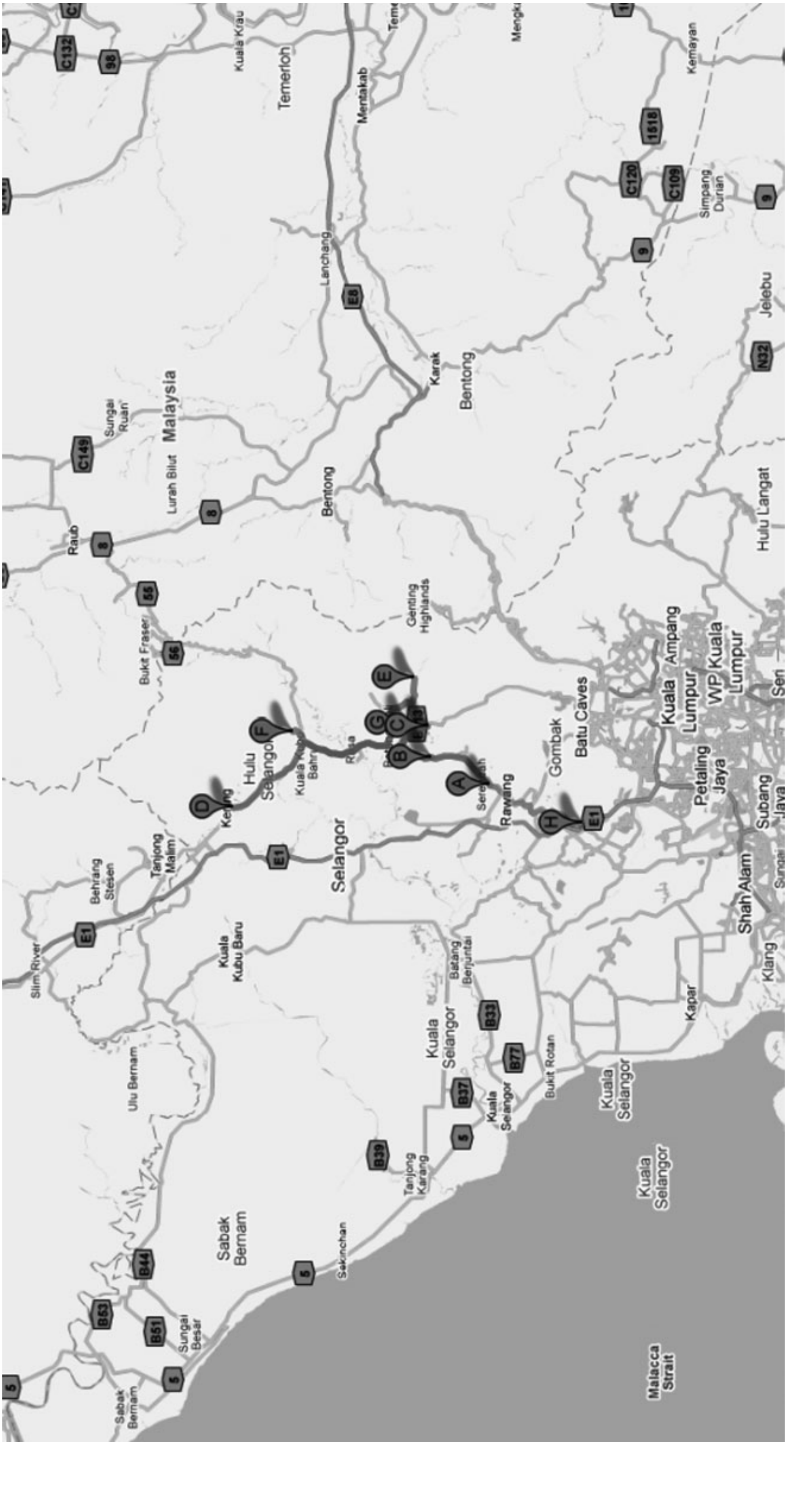

FIG. Map of Selangor state, showing the location of the eight study villages (labelled A–H).

Control and Prevention, Atlanta, GA). A child who had a *z*-score more than two S.D. below the corresponding median for the NCHS reference population was considered to be significantly malnourished.

Statistical Analysis

The collected data were analysed using version 11.5 of the SPSS for Windows software package (SPSS Inc, Chicago, IL). For the descriptive data, percentages were used to assess the prevalence of infection. Univariate analyses were used to investigate the association between *Cryptosporidium* infection and each independent variable investigated. Binary logistic regression was then used to explore the relative importance of each risk factor identified in the univariate analyses. A *P*-value of <0.05 was considered indicative of a statistically significant difference or association.

RESULTS

All 276 children (141 boys and 135 girls) aged 2–15 years who participated in the study appeared to be healthy and without diarrhoea. The median age of the children was 7 years (interquartile range=4–10 years). Informed consent for the inclusion of any child aged <2 years could not be obtained. Most (68.3%) of the children enrolled were from families with low household incomes (of <450 ringgits/month). The basic characteristics of the subjects are shown in Table 1.

Cryptosporidium oocysts were detected in the faecal samples of 20 (7.2%) of the children. Although the prevalence of *Cryptosporidium* infection was relatively high in the children aged <8 years old and higher among the girls investigated than among the boys (Table 2), these differences did not reach statistical significance. Although microsporidial spores were found in 57 (20.7%) of the 276 faecal samples, the microsporidial infections among the present subjects have already been described by Norhayati *et al.* (2007).

TABLE 1. *Characteristics of the 276 aboriginal children who participated in the study*

Characteristic	No. and (%) of children
AGE (years)	
2–4	66 (23.9)
5–7	83 (31.1)
8–10	71 (25.7)
>10	56 (20.3)
GENDER	
Male	141 (51.1)
Female	135 (48.9)
SOCIO-ECONOMIC STATUS	
Father educated for at least 6 years	202 (73.2)
Mother educated for at least 6 years	120 (43.5)
Low household income (<450 ringgits/month)	188 (68.1)
Working mother	43 (15.6)
Large household size (more than seven members)	108 (39.1)
NUTRITIONAL STATUS	
Significant underweight	156 (56.5)
Significant stunting	169 (61.2)
Significant wasting	42 (15.2)

The results of the univariate analysis (Table 3) indicated that a child was at significantly increased risk of *Cryptosporidium* infection if he or she had weighed $\leqslant 2.5$ kg at birth [odds ratio (OR)=2.99; 95% confidence interval (CI)=1.04–8.64;

TABLE 2. *Prevalences of (detected)* Cryptosporidium *infection among the 276 aboriginal children, split by age and gender*

	No. and (%) of children:	
Characteristic	Examined	Found infected
AGE (years)		
2–4	66	5 (7.7)
5–7	83	8 (9.6)
8–10	71	3 (4.2)
>10	56	4 (7.1)
GENDER		
Male	141	10 (7.1)
Female	135	10 (7.4)
All subjects	276	20 (7.2)

TABLE 3. *The results of the univariate analysis of potential predictors of* Cryptosporidium *infection among the 276 aboriginal children*

	% of the children found:			
Variable	Uninfected	Infected	Odds ratio and (95% confidence interval)	*P*
Age <7 years	53.1	65.0	1.60 (0.63–4.24)	0.305
Male	51.2	50.0	1.05 (0.42–2.60)	0.920
Birth weight ⩽2.5 kg	27.6	53.3	2.99 (1.04–8.64)	0.035
Breast fed for ⩽4 months	19.2	20.0	1.05 (0.34–3.29)	0.935
Prolonged breast feeding (>2 years)	4.9	15.2	2.18 (1.02–7.32)	0.029
Incomplete immunization	6.3	5.0	0.79 (0.12–6.28)	0.820
Significant underweight	54.8	55.6	1.03 (0.39–2.71)	0.940
Significant stunting	58.7	66.7	1.41 (0.51–3.88)	0.507
Significant wasting	14.8	21.4	1.58 (0.41–6.02)	0.503
Father's education poor (<6 years)	30.1	40.0	1.55 (0.61–3.94)	0.359
Mother's education poor (<6 years)	59.2	45.0	0.57 (0.23–1.41)	0.217
Low household income (<450 ringgits/month)	68.4	70.0	1.08 (0.40–2.92)	0.879
Large family size (more than seven members)	10.0	41.4	5.31 (1.37–14.43)	0.006
Working mother	14.6	15.0	0.97 (0.27–3.48)	0.960

P=0.035], had been breast-fed for >2 years (OR=2.18; CI=1.02–7.32; *P*=0.029) or lived in a household that had more than seven members (OR=5.31; CI=1.37–14.43; *P*=0.006). In the binary logistic regression (Table 4), however, living in a household with more than seven members was the only statistically significant predictor of *Cryptosporidium* infection that was detected (OR=2.15; CI=1.25–5.02; *P*=0.025). No significant association between *Cryptosporidium* infection and stunting, wasting or underweight was observed (Table 3).

DISCUSSION

Although the prevalence of *Cryptosporidium* infection detected in the present study, among aboriginal children in the south of the country, was 7.2%, it is clear that the prevalence of such infection varies in Malaysia, according to the population studied. In an earlier, community-based study, for example, Lai (1992) detected *Cryptosporidium* infection in 10.6% of children aged <7 years, whereas, in recent, community-based surveys carried out among all age-groups of aboriginals, such infection was detected in 4.1%–5.5% of the subjects (Mahdy *et al.*, 2007; Lim *et al.*, 2008). Prevalences varying from just 0.9% to 11% have been detected in hospitalized patients in Malaysia (Ludin *et al.*, 1991; Lai, 1992; Menon *et al.*, 2001).

In general, in most Asian countries, Europe and North America, between 1% and 3% of children appear to harbour *Cryptosporidium* infections at any one time (Cross *et al.*, 1985; Aye *et al.*, 1994;

TABLE 4. *The statistically significant results of the multivariate analysis of potential predictors of* Cryptosporidium *infection among the 276 aboriginal children*

Variable	Parameter estimate (B)	S.E. of estimate	Odds ratio and (95% confidence interval)	*P*
Constant	−1.140	0.501	–	0.000
Large household size (more than seven members)	0.764	0.201	2.15 (1.25–5.02)	0.025

Current, 1994; Katsumata *et al.*, 1998; Wang *et al.*, 2002). In Africa, however, the corresponding prevalence is generally much higher, at about 10% (Current, 1994). Any reported prevalences have to be treated with caution, however, as they can be greatly influenced by the number of faecal samples checked for each subject and the technique used for the detection of the protozoa — the use of concentration techniques, for example, usually increasing the number of checked subjects found positive for *Cryptosporidium* oocysts. Although the present results were based on just one sample/subject (because of limited resources and the cultural beliefs of the Orang Asli), the detection method employed — modified Ziehl–Neelsen staining (Henriksen and Pohlenz, 1981) — is specific and sufficiently sensitive to detect moderate–high numbers of *Cryptosporidium* oocysts in stool specimens (Garcia *et al.*, 1992; Quilez *et al.*, 1996). As light infections may have been missed, the prevalence estimated in the present study is, however, likely to be an under-estimate of the true prevalence of *Cryptosporidium* infection among the children who were investigated. Although some fungal spores have been found to be acid-fast (like *Cryptosporidium* oocysts), they can usually be distinguished from *Cryptosporidium* oocysts by size, the oocysts measuring 4–6 μm in diameter while fungal spores are generally larger (Casemore, 1991).

In the present study, the main risk factor for, or predictor of, *Cryptosporidium* infection among the children (and the only one found statistically significant by logistic regression) was living in a household that was relatively large (i.e. one with more than seven members). Children who lived in such large households were more than twice as likely to be found infected than their counterparts who lived in smaller households, probably because the larger households were more likely to be overcrowded, increasing the probability of person-to-person transmission. Most human infections with *Cryptosporidium* are the result of person-to-person transmission, especially in developing countries where standards of hygiene are poor (Samie *et al.*, 2006). When Garcia-Rodriguez *et al.* (1990) checked children attending Spanish day-care centres and primary schools, they only found *Cryptosporidium* infection in the infants (aged <3 years), attributing the infections to the relatively poor personal hygiene of, and high levels of person-to-person contact in, this age-group. Unfortunately, data on some factors that have previously been found to be associated with *Cryptosporidium* infection, such as animal contact and certain types of water supply (Berkelman, 1994; Hunter and Thompson, 2005), were not collected in the present study. The possibility of water-borne, food-borne and/or zoonotic transmission of *Cryptosporidium* in the present study villages still needs to be investigated.

Although many of the aboriginal children investigated in the present study showed stunting, underweight and/or wasting, none of these indicators of malnourishment was found to be significantly associated with *Cryptosporidium*. A significant association between *Cryptosporidium* and malnutrition has been detected in Peru (Checkley *et al.*, 1998), Brazil (Guerrant *et al.*, 1999) and Haiti (Kirkpatrick *et al.*, 2002) but mostly among younger children than those included in the present study. In their cohort study in Guinea-Bissau, West Africa, Molbak *et al.* (1997) found that the effect of *Cryptosporidium* on nutritional status declined with increasing age, older children possibly acquiring partial immunity that reduced the manifestations of *Cryptosporidium* infection. The malnutrition seen so frequently among the 'older' aboriginal children investigated in the present study is probably more the result of poverty and giardiasis than of *Cryptosporidium* infection, as indicated by the results of previous research in the same communities (Al-Mekhlafi *et al.*, 2005*a*, *b*).

In the present study, at least in the univariate analyses, prolonged breast-feeding and low birthweight were found to be significant predictors of childhood infection with *Cryptosporidium*. Curiously, these two factors have been widely documented as strong predictors of childhood malnutrition (Molbak *et al.*, 1994*b*; Simondon and Simondon, 1998; Tome *et al.*, 2007) but not, previously, of *Cryptosporidium* infection in childhood. In the villages investigated in the present study, prolonged breast-feeding and low birthweight may be leading to malnutrition that, in turn, may be increasing the susceptibility of local children to parasitic infections and/or decreasing the ability of the children to clear such infections. In mice experimentally infected with *C. parvum*, CD4 T-cells in the epithelium of the small intestine appear to play a major role in the clearance of the parasites (McDonald, 2000). In their review, Hughes and Kelly (2006) concluded that malnutrition-associated immunodeficiency not only occurs but also impairs a human's defences against parasitic infection.

In conclusion, cryptosporidial infection is probably a significant health problem among the aboriginal communities in Selangor, where most human infections with *Cryptosporidium* probably result from person-to-person transmission in overcrowded households. Although, currently, the *Cryptosporidium* in these communities does not appear to play a major role in the high prevalence of childhood malnutrition, the parasite is likely to gain in local importance as HIV infection becomes more common in the communities. Health education may help in controlling cryptosporidiosis in these communities, with special attention being given to the HIV-infected and other high-risk groups. *Cryptosporidium* detection should also be included in the routine examinations of faecal samples from the study villages (and similar communities).

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