A SEROEPIDEMIOLOGICAL STUDY OF TOXOCARIASIS AND RISK FACTORS FOR INFECTION IN CHILDREN IN SRI LANKA

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Abstract. A seroepidemiology study using TES-ELISA was carried out in 1,020 children aged 1-12 years in the Hindagala Community Health Project, Sri Lanka. Toxocariasis seroprevalence was 43% with 16.6% showing high antibody levels. Unconditional logistic regression analysis showed 7- 9 year olds to be at the highest risk (OR 3.0820; CI= 1.95-4.87). Dog ownership, especially puppies (OR 29.28; CI= 7.40-116.0), and geophagia-pica (OR 6.3732; CI= 3.87-10.50), were significant risk factors. Family clustering of toxocariasis was significant (χ^2 = 88.000; p= 0.0001). Abdominal pain (45%), cough (30%), limb pain (23%) and skin rashes (20%) were significantly associated with seropositivity indicating that toxocariasis causes covert morbidity. These findings are, overall, applicable to other areas in Sri Lanka. However, in the dry zone, survival of infective eggs in the soil could be affected by the climate while more importantly, in agricultural areas with a high buffalo population, *Toxocara vitulorum* could account for human toxocariasis. Using a species specific double sandwich ELISA based on 57 kDa protein of *T. canis* ES antigen, it is demonstrated that 91% of the seropositives were due to *T. canis*. Thus along with rabies and dirofilariasis, toxocariasis is an important zoonotic health hazard from dogs in Sri Lanka and prevention is indicated.

INTRODUCTION

Of the *Toxocara* spp infecting humans *Toxocara canis* and *Toxocara cati*, parasites of canines and felines respectively, are the most important in the domestic environment. Worldwide, the highest prevalence rates are in children (Gillespie, 1993). As humans are not the natural hosts the parasite fails to develop to maturity and is arrested in the larval stage. Diagnosis of toxocariasis is currently based on serology to detect antibodies. The widely used assay is the ELISA test based on the excretory-secretory antigens of second stage larvae derived from culture (TES-ELISA).

Seroprevalence of toxocariasis in children based on TES-ELISA has shown to vary up to 10% in temperate populations (Schantz, 1989) while in selected areas of low socio-economic conditions higher prevalence rates of 47% and 83% are documented (Radman *et al*, 2000; Thompson *et al*, 1986). However, from developing countries where parasitic infections, especially those with intestinal nematodes, is common in children, studies are limited. Childhood infection has been associated with pica and pet ownership (Glickman *et al*, 1981; Marmor and Glickman, 1987). Poor sanitation, and lack of health awareness are other risk factors identified (Thompson *et al*, 1986). In Sri Lanka, apart from a few case reports, the prevalence of infection in the community has not been studied. Although the accepted clinical manifestations in toxocariasis are visceral larva migrans (VLM) and ocular disease, pediatricians, even in high exposure areas, rarely encounter these typical syndromes. Thus there is concern that much of childhood morbidity due to toxocariasis is unrecognized.

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MATERIALS AND METHODS

Study area (HCHP)

This study was conducted in the Hindagala

Community Health Project (HCHP). HCHP covers an area of 39 km^2. It is mainly rural and is located in the Central Province of Sri Lanka in proximity to the University of Peradeniya. Topographically it consists of hills and low-lying valleys and includes paddy cultivation, agricultural settlements and tea estates (elevation 500 to 1,200 m). The HCHP has 16 villages in 7 midwife areas. Seven study sites, namely Kalugamuwa, Gurukele, Doluwa, Nillambe, Palle Deltota, Haloya and Mahakanda, were selected to represent the varied geographical terrain, socio-economic level and agricultural base (Fig 1). Of these seven areas, Palledelthota, and Haloya are mainly hilly tea plantation areas, Mahakanda, Kalugamuwa and Doluwa are semi-urban areas situated in the valley while Gurukele and Nillambe, are more rural. Overall, the socio-economic status of the community was low and the educational status of the adult population was poor. The population was 19,122 and 5,440 were in the age group of 1 -14 years. The male to female ratio in this group was approximately 1:1. Of the total population, 77.6% were Sinhalese, 19.9% were Tamils and the rest included Moors and other groups (Fernando, 1993).

Study population

The study population consisted of 1,020 children between 1-12 years of age from the seven study sites. This comprised 18.7% of the total aged 1 -14 years. The group included 988 Sinhalese and 32 Tamils (from tea estate lines).

Specimen collection and processing

Parents or guardians were contacted through the public health worker of the area and following preliminary briefing they were instructed to bring their children to the examination center in the area on a specified Saturday. Five to eight examination-visits were carried out in each area. Individual records for each child were made and included clinical symptoms relating to the disease and risk factors for toxocariasis. Pet ownership was defined as the presence of a pet dog, pups (less than 3 months) or cat in the home for at least 3 months. Information on worm treatment of pets and on disease awareness was included.

Collection of blood

Ethical clearance was obtained from the Committee on Research and Ethical Clearance, Faculty of Medicine, University of Peradeniya. Following written consent from the parents or guardian, venous blood samples were collected and the separated serum was stored at -20ºC.

Serodiagnosis by ELISA using *Toxocara canis* larval excretory-secretory antigen (TES-ELISA)

The TES-ELISA was carried out on the children's sera as described by De Savigny *et al* (1979) using microtiter plates coated with ES antigen obtained on a 4-week *in vitro* culture of *T. canis* infective larvae. The optical density (OD) values were determined photometrically at 490 nm in an ELISA reader (MINIREADER 11, Dynatech Laboratories Inc). Each of the test sera was tested in duplicate and the mean absorbance was taken. Interpretation of ELISA was based on OD values determined for local population (Wijesundera, unpublished data) as follows: 0.2 = no serological evidence of toxocariasis; 0.2 - 0.7 = compatible with past infection or current light infection and >0.7 = compatible with recent infection.

Double sandwich ELISA using *T. canis* species specific antigen (TcES-57 double sandwich ELISA)

All serum samples positive by TES- ELISA were tested with the TcES-57 double sandwich ELISA developed with a species specific protein of 57 kDa obtained on SDS-PAGE (Iddawela, unpublished data).

Statistical analysis

The data was entered in a Microsoft Excel data sheet. After checking the consistency of the database, it was transferred to a SPSS data sheet for analysis. Initially the seroprevalence of toxocariasis was calculated for the whole population and then for the specific age categories. The distribution of the clinical symptoms and their associations to the seropositivities was assessed using chi-square statistics.

The risk assessment was done in terms of odds ratios. Bivariant analysis calculated the

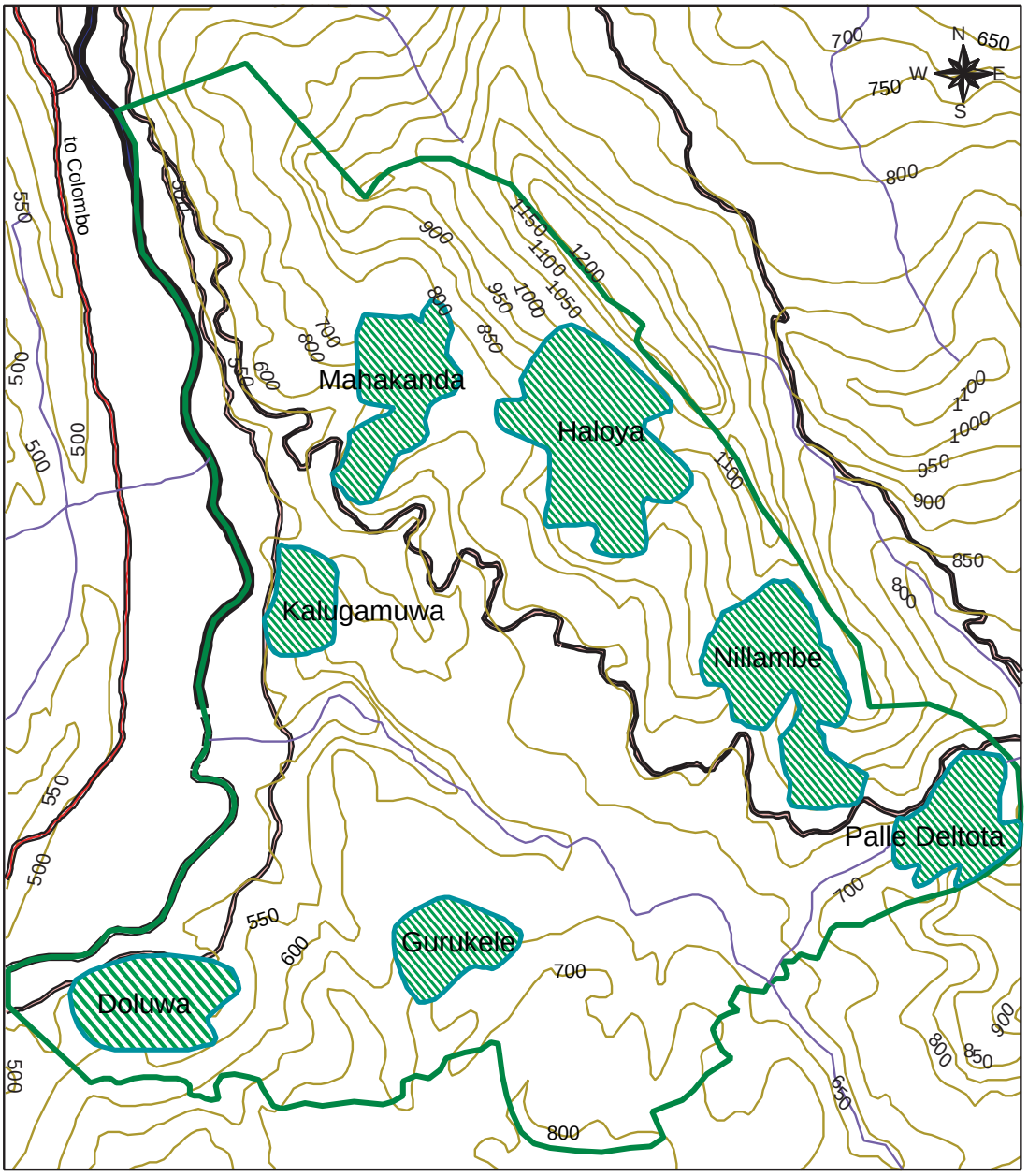

Fig 1–Study sites in the Hindagala Community Health Project (HCHP) – Location and topography.

crude odds ratios (Schlesselman and Stolley, 1982), following which the significant variables were fitted to the unconditional logistic regression model to eliminate the possible confounding factors and the adjusted odds ratios were calculated (Kleinbaum, 1990).

The dependent variable (ELISA seropositivity) and the independent variables were coded as follows:

(a) ELISA
- seronegative = 0; seropositive = 1

(b) Age group
- 1 –3 y = 0; 4 - 6 y= 1; 7 – 9 y = 2; 10 –12 y = 3

(c) Dog ownership
- no dogs = 0; adult dogs = 1; pups = 2

(d) Pica
- no pica = 0; pica + = 1

(e) Sex
- male = 1; female = 0

(f) Socio-economic status
- high =0; moderate = 1; low = 2

The socio-economic status of the household was grouped on the parental or guardian's occupation, into 3 categories *viz* unskilled labor (low), skilled labor (moderate) and as others, professionals (high).

RESULTS

TES - ELISA seropositivity in the study group

Overall, seropositivity was 43.2% (441). Of which 172 (16.6%) were positive at high level of *Toxocara* antibody carriage (OD >0.7; compatible with recent infection) and 269 (26.6%) were positive at low levels (compatible with light infection or past exposure).

TES - ELISA seropositivity by age

The highest prevalence of seropositivity was in the children aged 7 - 9 years (56.8%). While the lowest prevalence was in the 1-3 year age group (30.7 %) (Table 1).

TES - ELISA seropositivity in different study sites

The overall seropositivity varied in the different study sites (Table 2). Haloya showed the highest percentage (54.3%) and the lowest percentage of seropositivity was from the Mahakanda (34%).

TES-ELISA seropositivity in the estate sector

In the estate Tamil population, 75% (24) were positive for *Toxocara* ELISA antibody carriage. In the Sinhala population, the seropositivity prevalence was 41.4% (409).

De-worming and toxocariasis awareness

In this study group, every child had been de-wormed with mebendazole at the family health clinic at least once. None of the parents or guardians (>90% females) was aware of toxocariasis as a human disease and its usual mode of transmission to humans. Of the pet dogs, 95% were never de-wormed and the rest were also not regularly de-wormed.

Table 1
Distribution of seroprevalence by age groups.

Age group	No. surveyed	ELISA (+)	% Positivity
1 - 3	264	81	30.7
4 - 6	324	129	39.8
7 - 9	304	172	56.6
10 - 12	128	59	46.1
Total	1,020	441	43.2

Table 2
Distribution of seroprevalence by study site.

Study site	No. surveyed	ELISA (+)	% Positivity
Mahakanda	147	50	34.0
Doluwa	50	18	36.3
Kalugamuwa	228	87	38.2
Gurukele	43	18	41.9
Palle Deltota	212	91	42.9
Nillambe	108	51	47.2
Haloya	232	126	54.3
Total	1,020	441	43.2

Association of seropositivity with risk factors by bivariant analysis

The 7-9 year group was at the highest risk and was highly significant while sex and socio-economic status were not significant as risk factors (Table 3). Of the 7 study sites, Haloya and Nillambe were significant risk factors for seropositivity. Households owning both dogs and cats had the highest risk and were highly significant. Dog ownership was also highly significant while those owning pups had the highest risk. Cat ownership was not found to be a significant risk factor. Pica for geophagia was found to be significant.

Overall significance of risk factors

Unconditional logistic regression module

Table 3
Unadjusted odds ratios of risk factors for toxocariasis on independent variables.

Variable	Categories	ELISA (+)	ELISA (-)	OR	CI
Age (years)	1 - 3[a]	81	183	1	
	4 - 6	129	195	1.49	1.06 - 2.11
	7 - 9	172	132	2.90	2.05 - 4.11
	10 - 12	59	69	1.93	1.25 - 2.98
Sex	Female[a]	253	306	1	
	Male	189	274	0.82	0.64 - 1.05
SEC group	High[a]	101	130	1	
	Moderate	40	70	0.74	0.47 - 1.19
	Poor	300	379	1.04	0.77 - 1.41
Study site	Mahakanda[a]	50	97	1	
	Doluwa	18	32	0.93	0.48 - 1.81
	Kalugamuwa	87	141	1.18	0.76 - 1.83
	Gurukele	18	25	1.38	0.69 - 2.77
	Palle Deltota	91	121	1.44	0.93 - 2.24
	Nillambe	51	57	1.7	1.03 - 2.86
	Haloya	126	106	2.28	1.48 - 3.51
Pet ownership	No pets[a]	63	388	1	
	Dogs	296	107	17.31	12.23 - 24.51
	Cats	11	67	1.01	0.51 - 2.02
	Cats and dogs	71	17	26.14	14.44 - 47.3
Dog ownership	No dogs[a]	72	453	1	
	Adult dogs	282	120	14.8	10.68 - 20.57
	Pups	86	06	90.37	30.09 - α
Pica	Absent[a]	303	545	1	
	Present	138	34	7.4	4.92 - 10.96

[a]Reference category

identified dog ownership, age and pica as the most important independent risk variables of all independent variables considered, for toxocariasis (Table 4). Cat ownership and study sites were not included as these were not significant or only marginally significant on bivariant analysis. Thus the variables sex and socio-economic status of the study group can be considered as confounding factors.

Seropositivity and family clustering

The families with two or more children were grouped into three *viz* those having two or more seropositive siblings (17), those with only one seropositive sibling (09) and those families where the siblings were seronegative

Table 4
Adjusted odds ratios of risk factors for toxocariasis on independent variables.

Variables	Category	Adjusted OR[b]	95% CI
Dog ownership	No dogs[a]	1	
	Adult dogs	15.04	10.57 - 21.40
	Pups	29.28	07.40 - 116.0
Age	1 - 3[a]	1	
	4 - 6	1.73	1.10 - 2.72
	7 - 9	3.08	1.95 - 4.87
	10 - 12	2.61	1.46 - 4.67
Pica	Absent[a]	1	
	Present	6.37	3.87 - 10.50

[a]Reference category
[b]Odds ratios are controlled for all other variables using the unconditional logistic regression model.

Table 5
Association between clinical features and TES-ELISA.

Clinical feature		ELISA +ve (%)	ELISA -ve (%)	χ^2	p-value
Abdominal pain	Present	198 (45)	30 (5.2)	227.511	<0.0001
	Absent	243 (55.1)	549 (94.8)		
	Total	441 (100)	579 (100)		
Anorexia	Present	176 (40)	25 (4.3)	200.415	<0.0001
	Absent	265 (60.1)	554 (95.7)		
	Total	441 (100)	579 (100)		
Cough	Present	132 (30)	20 (3.5)	138.394	<0.0001
	Absent	309 (70.1)	559 (96.5)		
	Total	441 (100)	579 (100)		
Limb pain	Present	100 (22.7)	30 (5.2)	68.894	<0.0001
	Absent	341 (77.3)	549 (94.8)		
	Total	441 (100)	579 (100)		
Skin rash	Present	90 (20.4)	23 (4.0)	68.646	<0.0001
	Absent	351 (79.6)	556 (96.0)		
	Total	441 (100)	579 (100)		
Pruritus	Present	60 (13.6)	20 (3.5)	35.689	<0.0001
	Absent	381 (86.4)	559 (96.5)		
	Total	441 (100)	579 (100)		
Loss of weight	Present	50 (11.3)	10 (1.7)	41.765	<0.0001
	Absent	391 (88.7)	569 (98.3)		
	Total	441 (100)	579 (100)		
Fever	Present	20 (4.5)	2 (0.3)	20.823	<0.0001
	Absent	421 (95.5)	577 (99.7)		
	Total	441 (100)	579 (100)		
Headache	Present	20 (4.5)	5 (0.9)	14.114	<0.0001
	Absent	421 (95.5)	574 (99.1)		
	Total	441 (100)	579 (100)		
Vomiting	Present	10 (2.3)	1 (0.2)	10.298	0.001
	Absent	431 (97.7)	578 (99.8)		
	Total	441 (100)	579 (100)		
Convulsions	Present	4 (0.9)	1 (0.2)	2.767	0.096
	Absent	437 (99.1)	578 (99.8)		
	Total	441 (100)	579 (100)		

(18). A significantly high prevalence (χ^2 = 88.000; p =0.00001; df = 4) of seropositivity was seen in the group where two or more siblings were seropositive.

TES - ELISA seropositivity and clinical symptoms

The clinical symptoms in the seropositives and the seronegative groups are analysed in Table 5. The major symptoms: abdominal pain, anorexia, cough, limb pain and skin rash were significantly associated with seropositivity. Five children gave a history of convulsions. In 4 of the children, the convulsions were febrile fits while the remaining child had been diagnosed and was under treatment for epilepsy. In this group, 4 were seropositive and 1 child with febrile fits was seronegative.

Validation of seropositivity on TcES-57 double sandwich ELISA

Of the 441 TcES- ELISA positive serum

samples, 9% (40) were negative on the double sandwich ELISA using TcES-57. When high and low positive antibody levels were considered, 5% of those with high levels were negative while 7% of the low positives were negative on this ELISA.

DISCUSSION

This is the first seroepidemiological study on toxocariasis in a Sri Lankan population. The positive antibody carriage of 43% for toxocariasis in the 1-12 year age group indicates that infection is common. In 16.6%, a high level of seropositivity was seen implicating recent or heavy infection. This pattern of TES antibody carriage is comparable to that reported by Radman *et al* (2000) in children (younger than 15 years) in La Plata, Argentina but is much lower than the 86% prevalence in a rural Colombian study where the children were from a very low socio-economic level (Thompson *et al,* 1986). A Venezuelan study comparing urban and rural communities reported an overall 65% prevalence rate for all ages (Lynch *et al,* 1988). The same study considering the seropositivity at the high cut-off titer (similar to value used in this study) showed 25.6% for the rural farming community.

The age of a typical VLM patient is a child between the ages of 2-7 years (Glickman and Schantz, 1981). However, in this study, positive *Toxocara* antibody carriage was highest in children aged 7-9 years (56.8%) while the lowest prevalence was in the 1-3 year group. Age group related seropositivity studies from developing countries are few. The Colombian study (Thompson *et al,* 1986) was confined to the younger children (6 month-6 years) while the La Plata study (Radman *et al,* 2000) reported a higher positivity of 47% in children less than 15 years when compared to adults but distribution within the childhood age group was not reported. No significant association was found between sex and seropositivity.

Infection, as indicated by seropositivity, showed clustering among families with 60% having more than one sibling affected. Holland *et al* (1991) showed household clustering in toxocariasis in Sweden. In soil-transmitted helminthiasis such as ascariasis, overdispersion of infection in households is well recognized (Crompton and Tulley, 1987). Thus family clustering of toxocariasis is not surprising and implies that household hygiene and socio-economic status has a bearing on acquisition of infection.

Toxocariasis is reported to be high among inhabitants of rural areas with low educational and socio-economic status (Lynch *et al,* 1988; Genchi *et al,* 1990; Havasiova *et al,* 1993). Even in developed countries rural children are shown to have a significantly higher prevalence (Embil *et al,* 1988). The HCHP area is mainly rural with a few semi-urban foci. The socio-economic standards of the study group were in general low. Of the 7 study sites, Mahakanda is a semi-urban site and showed, when compared to the other sites, a significantly lower percentage of seropositivity. The comparatively higher level of parental education and socio-economic status could explain this. The estate Tamil population having a low education level, socio-economic status and personal hygiene, not surprisingly showed a very high prevalence (75%). However, valid conclusions on difference in prevalence in this sector cannot be drawn as the estate sector was highly underrepresented. Overall, the socio-economic standard was not significant as a risk factor for toxocariasis in this study.

Within the study area two sites, namely Haloya and Nillambe, showed a higher prevalence which was significant. The former includes several tea estates while in Nillambe contaminated soil dispersion is more likely, as it is a hilly terrain with habitations both on the hills and valleys.

Of the risk factors evaluated in this study, dog ownership had a highly significant association with seropositivity. The association was most significant with ownership of puppies (less than 3 months old). It is widely reported that *T. canis* is most prevalent in pups. In experimental infections, highest worm loads occur in

puppies less than on-month old (Lloyd, 1993).

When age was analysed as a risk factor, the 7-9 year age group was shown to be at most risk to toxocariasis. In Sri Lanka, especially in the rural areas, the very young child is better cared for and is generally looked after inside the house. While the young schooling child is more independent, they often spend much of their time playing outdoors in the school playground or garden. Personal hygiene is minimal with hand washing before meals, an unlikely habit to be adhered to and generally frowned upon, in this age group. Picking and eating fruits fallen on the ground is also a common habit relished by the rural child. These are possible explanations for the higher risk exposure in this age group in our environment. It is noteworthy that the risk increased with age reaching a peak at the 7-9 year olds but then onwards decreased sharply in the older child of 10-12 years. Decreased exposure due to change in behavior patterns and better personal hygiene is the likely reason, although development of immunity with age cannot be ruled out. Geophagia- pica was a significant risk factor but was not as common as shown by Huntly *et al* (1965).

The pathogenesis of toxocariasis is attributed to the mechanical damage produced by larvae migrating through viscera and to the degree of the host immune response. Clinical manifestations are thus variable and depend on the number of infective eggs ingested (Glickman and Schantz, 1981). Agudelo *et al* (1990) were unable to correlate clinical features, defined as VLM, with *Toxocara* seropositivity. However, toxocariasis is suspected to be responsible for other common childhood morbidity as shown by covert toxocariasis. Taylor *et al* (1988) showed that non-specific symptoms could be attributed to toxocariasis forming a symptom complex. The major symptoms identified by these authors included abdominal pain, anorexia, nausea and cough.

This study further supports the hypothesis that a high proportion of idiopathic abdominal pain of childhood is due to toxocariasis. Abdominal pain could be due to mesenteric lymphadenitis as a host response to the migrating larvae. In experimental studies in puppies intestinal villi showed increase in immune cell activity associated with larval migration in tissues (Lloyd *et al*, 1991).

In agreement with Buijs *et al* (1994) and Radmen *et al* (2000), respiratory symptom such as cough was common among seropositive children. Although the natural history and host reaction to the migrating second stage larvae within the human lungs is not known, experimental infections in mice has shown multifocal inflammation in the lungs (Buijs *et al*, 1990). Neurological symptoms were rare in this study. A recent clinical study in Sri Lanka has focused on ecchymoses as a clinical presentation in toxocariasis (Wickremasinghe *et al*, 2001). The significant association of several symptoms with seropositivity indicates the need to recognize covert toxocariasis as a disease entity in children.

When assaying the seropositives for *T. canis* species specificity, it is noteworthy that 91% were positive for *T. canis* on the species-specific antigen test, TcES-57 double sandwich ELISA developed in this laboratory. Thus confirming *T. canis* to be the most important etiological agent of toxocariasis in this area. However, 9% of the seropositives were not picked up by this test. When the high cut-off titer was considered, 4% tested negative while at the low cut-off titer, a higher percentage of 7% were negative. The widely used TES-ELISA is not species specific and gives positive results with other *Toxocara* species such as *T. cati* of cats and *T. vitulorum* of cattle and buffalos. Thus, in the Sri Lankan environment *T. cati* and *T. vitulorum* could probably account for the seropositivity not detected in the species-specific test. However, in this study site buffalos were uncommon. Although species-specific diagnostic tests for *T. canis* and *T. cati* based on ES antigens have been researched and developed using recombinant technology, these have not been evaluated as yet in determining the species-specific etiology in toxocariasis (Yamasaki *et al*, 2000) while a reliable diagnostic test specific for *T. vitulorum* is yet to be developed.

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