SERO-EPIDEMIOLOGY OF *TOXOCARA CANIS* INFECTION IN CHILDREN ATTENDING FOUR SELECTED HEALTH FACILITIES IN THE CENTRAL REGION OF GHANA

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Conflict of Interest: *None declared*

SUMMARY

Objective: The study determined the seroprevalence of *Toxocara canis* infection among children attending four selected health facilities in the Central Region of Ghana.

Design: Cross-sectional study

Method: Sera from 566 children aged 1-15 years attending four selected health facilities in the Central Region of Ghana between July and September 2012 was used in a Toxocara excretory-secretory antigen-based ELISA to detect serum IgG. A short questionnaire was designed to obtain data on respondents as to age, gender, educational level, locality of residence, habits of washing of fruits, vegetable and hands before eating, keeping of pet (dogs or cats) , and history of playing with soil and pets. Clinical information was also collected. Associations between sero-positivity and age group, gender, risk factors, educational level and other variables were determined by Chi square test.

Results: The overall sero-prevalence was 53.5% (n=566). Age, educational level and hospital visited were significantly associated with sero-positivity ($p< 0.05$). Children with history of playing with soil ($\chi^2=9.03$, $p=0.003$), pet-keeping ($\chi^2=14.77$, $p=0.001$) and not washing hands with soap before eating ($\chi^2=5.82$, $p=0.016$) were significantly associated with sero-positivity.

Conclusion: The sero-prevalence of *T. canis* infection in children in the study was high. The children should be educated to desist from risk factors such as playing with soil and pets and be encouraged to ensure proper personal hygiene.

Keywords: Seroprevalence, Toxocariasis, risk factors, children, Ghana

INTRODUCTION

Human toxocariasis is a zoonosis caused by larval stages of *Toxocara canis* and, less frequently, by *T. cati*, the roundworms of dogs and cats, respectively.[1] The adult worm lives in the small intestines of definitive hosts (cats and dogs) where it produces eggs that pass out with the faeces.[2] The eggs are unembryonated when freshly passed with faeces into the environment.[3] Under optimal temperatures and humidity, these eggs develop into embryonated eggs that are infectious to both definitive and paratenic hosts.[1]

Humans become infected when they accidentally ingest embryonated *Toxocara* eggs containing the infective larvae. These parasites cannot develop into adult forms in humans and are restricted to larval forms, migrating through the soft tissues for months and even years and causing local or systemic inflammatory reactions in the affected organ.[4] Symptoms shown by infected persons depend on the organs affected and the magnitude of infection[5] resulting in several clinical forms of human toxocariasis. However, Smith and colleagues consider that human toxocariasis should be classified in three major forms: visceral larva migrans, ocular toxocariasis, and covert toxocariasis.[6]

The diagnosis of toxocariasis in humans depends heavily on serological test, as neither eggs nor larvae occur in the faeces.[5] The method of choice for diagnosis is excretory-secretory antigen-based enzyme-linked immunosorbent assay (ELISA) [7] which shows 78.3% sensitivity and 92.3% specificity.[8] Young children are the main population-at-risk for *T. canis* infection due to geophagia, pica, poor hygiene, or frequent contact with dogs.[2]

The prevalence of toxocariasis is generally higher in tropical and developing countries than in developed countries and has been associated with risk factors including playing in the soil and with dogs.[9, 10]

Reported prevalences range from 1% in Spain[11] to 86% in Santa Lucia.[9] Very little is known about the seroprevalence of toxocariasis in Africa and particularly in Ghana. This study was done to determine the seroprevalence of *Toxocara canis* infection and its associated risk factors in children (1-15 years of age) visiting four selected health facilities in the Central Region using TES- ELISA.

METHOD

Study sites

This study was conducted in hospitals in the Central Region of Ghana, located between latitudes 5°1'N and 6°18' N and longitudes 0°22'W and 2°10'W.[12] The region has an estimated population of 2,201,863 (2010 projection) and an annual population growth rate of 2.1%.[13] The major economic activities are fishing and agriculture. The region is classified among the four poorest in the country.[12]

The health facilities used were the University of Cape Coast Hospital in Cape Coast Metropolis, Twifo-Praso District Hospital in Twifo/Heman/Lower Denkyira District, St. Francis Xavier Hospital in Assin North Municipality and Elmina Health Centre in Komenda/Edina/Eguafo/Abrem District. The catchment population of these health facilities were people in the specific and surrounding districts.

Study population and ethical approval

A cross-sectional study design was used. The protocol for this study was reviewed and approved by Ghana Health Services Ethical Review Committee on Research Involving Human Subjects (ERCRIHS) and Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB), University of Ghana.

Children visiting the selected hospitals were recruited after obtaining informed verbal consent from their parents or guardians. Each child or the parent was interviewed to provide information on demographics and risk factors and symptoms of toxocariasis.

A total sample size of 560 was estimated using a formula by Fishers and colleagues.[14] In the absence of published prevalence for Ghana, a prevalence of 21.5% for Nigeria[15] was used as an estimate. In all 566 participants, spread across all study sites, were recruited for the study from July to September 2012.

The Inclusion criteria wereChildren between the ages of 1-15 years attending any of the four study site.
Children within the stated age group who were referred to the laboratories of any of the four study site.
Children whose parents agreed to allow them participate in the study. The Exclusion criterion was a child outside 1-15 years age range.

Blood Sample collection and ELISA

Two (2) ml of blood from each child was collected by venipuncture. The blood sample was dispensed into appropriately labelled tubes containing clot activator, centrifuged at 2500 rpm for 10 min to separate the serum and stored at -80°C (Ultra low temperature freezer, model MDF-U40865, Sanyo Electric Co. Ltd, Japan) until further used.

Serum-specific IgG antibodies were detected by an ELISA based on the method of Jimenez and colleagues[22] with slight modifications. Briefly, wells of microtitre plates (Greiner Labbortechnick, Germany) were coated with 50µl of TES antigen at 2µg/ml in coating buffer (34.5mM $NaHCO_3$ and 15.1mM $NaCO_3$, pH 7.4) and left for overnight incubation at 4°C.

The plates were then washed three times with 200µl of 0.05% Tween 20 PBS (Wako, Tokyo, Japan) and blocked with 200µl of blocking buffer (5% Casein) for 1 hour at 37°C to avoid non-specific binding. The plates were then washed five times with 200µl of the 0.05% Tween 20 PBS washing buffer.

Test serum, diluted 1:50, was added to each individual well and incubated for 90 minutes at 37°C. The optimal dilutions were determined by a checkerboard titration in previous optimization checks. Positive and negative controls (donated by Dr I Ayi, Accra, Ghana) were also diluted in the same way and included in each plate.

The last two columns (columns 11 and 12) of each plate were reserved for positive, negative and blank controls. Duplicate tests were run on each test serum as well as the controls. The plates were then washed five times with 200µl of the 0.05% Tween 20 PBS washing buffer.

Fifty micro litres (50µl) of horse radish peroxidase-conjugated goat anti-human IgG (Sigma Aldrich Company, St Louis MO, USA), diluted to 1:2500 in PBS, was added to each well and incubated for 1 hour.

The plates were then washed five times with 200µl of 0.05% Tween 20 PBS washing buffer. One hundred microlitres (100µl) of 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) solution (KPL, Maryland, USA) were added to each well and incubated for 30 minutes in the dark. The reaction was stopped by the addition of 1% sodium dodecyl sulfate (SDS) to each well.

The plates were then read at 415 nm wavelength with ELISA plate reader (Multiskan Ascent, model V 1.24 354-00978, Themo Lab Systems, Japan). A test serum whose mean optical density (OD) value was equal to or higher than the mean OD value plus three times standard deviations (SD) of the negative control serum was considered positive.

Socio-demographic Information

A short questionnaire to assess risk factors was designed to obtain data on respondents as to age, gender, educational level, locality of residence, habits of washing of fruits, vegetable and hands before eating, keeping of pet (dogs or cats) , and history of playing with soil and pets. Information on whether the children had had symptoms such as coughing, fever, body rashes, vomiting, stomach discomfort, difficulty in seeing, nausea and red eye was also obtained.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 16 for Windows and Minitab, version 15.1.20.0. The data on demography, risk factors and symptoms were summarised as frequencies. Chi square test was used to determine whether or not there were associations between sero-positivity and age group, gender, risk factors, educational level and other variable.

Multiple Logistic Regression (Multivariate-adjusted Odds Ratio) with 95% confidence intervals (CIs) was used to further analyze the various categories in age, educational level and health facility visited by the study children which had showed overall significant differences. The Odds ratio (OR) of a referent category was adjusted to unity (1). Significance was tested at $\alpha = 0.05$.

RESULTS

Sero-positivity was detected in 303 (53.5%, n=566) serum samples from children visiting four selected health facilities in the Central Region of Ghana. Significant differences were seen in prevalence based on age group, educational level and health facility visited, but not on the basis of gender or location of hospital (Table 1).

An analysis of the sero-prevalence of male and female children within the three age groups revealed a significant (p=0.024) difference in the sero-prevalence in males (47.3%, n=55) and females (66.3%, n=89) in the 11-15 year group but not within year groups 1-5 years (males 48.6%, n=152 versus females 45.1%, n=113; p = 0.567) and 6-10 years (males 61.7%, n=85 versus female 62.2% n=72; p = 0.865). Similarly, sero-positivity seemed to significantly increase as children progressed in the educational level (Table 1).

Table 1 *Toxocara canis* sero-positivity and characteristics of children attending four selected hospitals in the Central Region, Ghana

Variable Group (Characteristic)	No. Tested	No. Positive (%)	χ^2	*p*
Gender				
Male	292	154 (52.7)	1.53	0.696
Female	274	149 (54.4)		
Age group				
1-5 years	265	125 (47.2)	9.04	0.011[a]
6-10 years	157	97 (61.8)		
11-15 years	144	81 (56.3)		
Educational Level				
Not schooling	46	12 (26.1)	21.43	0.001[a]
Crèche	100	47 (47.0)		
Kindergarten	131	73 (55.7)		
Primary	182	112(61.5)		
Junior High School	107	60 (56.1)		
Health facility visited				
St. Xavier Hospital	156	85 (54.5)	8.89	0.031[a]
U C C Hospital	163	72 (44.2)		
Elmina Health Centre	147	87 (59.2)		
Twifo Praso Gov. Hosp.	100	59 (59.0)		
Location of hospital				
Coastal	310	159 (51.3)		
Hinterland	256	144 (56.3)	1.39	0.239

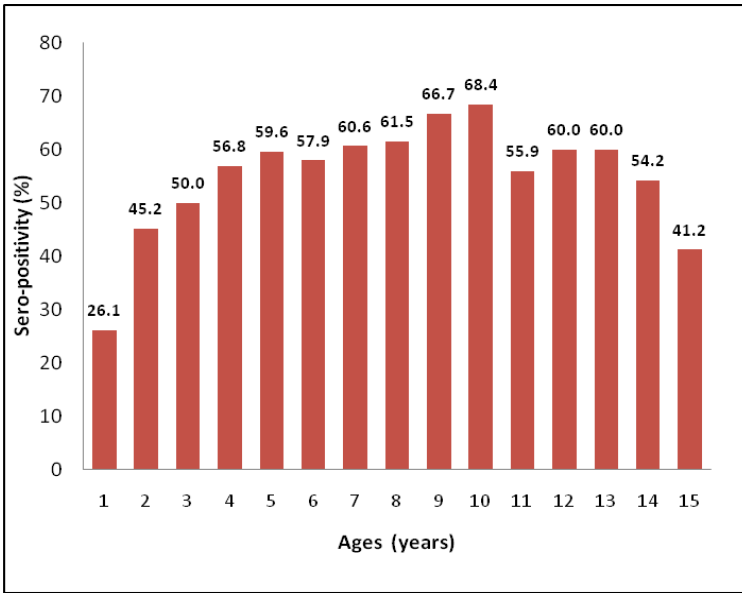

Figure 1 Relationship between age and sero-positivity

From Figure 1, sero-positivity seemed to increase with age. Children of 1 year old had lowest value (26.1%) rising to a peak at age 10 (68.4%) then declining to 41.2% at age 15.

The ORs were significant for the 6-10 year age group, children in kindergarten (KG), primary or junior high schools (JHS), and for those visiting Elmina Health Centre and Twifo-Praso Government Hospital, compared to the respective referent categories (Table 2).

Table 2 Multivariate-adjusted odds ratios for various characteristics associated with sero-positivity of *Toxocara canis* among children in Central Region of Ghana

Variable Group	No. Tested	No. Positive (%)	Multivariate adjusted OR (95% CI)	P
Age				
1-5	265	125 (47.2)	1.00† (referent)	
6-10	157	97 (61.8)	1.81 (1.21-2.71)	0.040
11-15	144	81 (56.3)	1.44 (0.46-1.04)	0.080
Educational level				
Not schooling	46	12 (26.1)	1.00¶ (referent)	
Crèche	100	47 (47.0)	2.21 (1.01-4.87)	0.049
K G	131	73 (55.7)	3.43 (1.59-7.43)	0.002
Primary	182	112 (61.5)	4.36 (2.06-9.26)	0.001
Junior High School	107	60 (56.1)	4.40 (1.58-7.67)	0.002
Health facility visited				
UCC Hospital	163	72 (44.2)	1.00§ (referent)	
St. Xavier Hospital	156	85 (54.5)	1.51 (0.97-2.35)	0.066
Elmina Health Centre	147	87 (59.2)	1.83 (1.17-2.88)	0.009
Twifo Praso Gov. Hospital	100	59 (59.0)	1.66 (1.09-3.01)	0.020
Combination of 3 Hospitals	403	231 (57.3)	1.72 (1.19-2.47)	0.004

† Adjusted variables other than age group,
¶ Adjusted variables other than educational level
§ Adjusted variables other than health facilities visited

There were significant differences between the sero-prevalences of those who kept pets and those who did not and also between those playing with soil compared to those who did not. Those who had a habit of washing their hands with soap and water before eating recorded significantly lower sero-positivity than those who did not (Table 3).

Table 3 Sero-positivity and various risk factors for Toxocariasis in children visiting four selected health facilities in the Central Region of Ghana

Risk factor	No. Tested	No. Positive (%)	χ^2	*p*
Keeping of pets (dog or cat)				
Yes	155	83 (62.5)	14.27	0.001
No	318	148 (46.5)		
Playing with pet(s)				
Yes	132	80 (60.6)	3.46	0.063
No	434	223 (51.4)		
Playing with soil				
Yes	341	200 (58.7)	9.03	0.003
No	225	103 (45.8)		
Habit of washing fruits and Vegetable before eating				
Yes	502	269 (53.6)	0.05	0.945
No	64	34 (53.1)		
Habit of hand washing with Soap before eating				
Yes	299	141 (47.2)	5.82	0.016
No	267	133 (57.3)		

Sero-positivity in children who reported of having coughs or difficulty in seeing well were significantly higher when compared to those without these symptoms (Table 4).

Table 4 *Toxocara canis* sero-positivity and signs and symptoms reported by children attending four selected hospitals in Central Region, Ghana

Signs and symptoms	No. Tested	No. Positive (%)	χ^2	*P*
Coughing				
Yes	293	171 (58.4)	5.69	0.017
Body rashes				
Yes	197	110 (55.8)	0.65	0.422
Difficulty in seeing clearly				
Yes	71	49 (69.0)	7.82	0.005
Red eye				
Yes	143	83 (58.0)	1.56	0.211
Vomiting				
Yes	245	130 (53.1)	0.04	0.844
Weight loss				
Yes	325	178 (54.8)	0.47	0.494
Loss of appetite				
Yes	318	167 (52.5)	0.30	0.582
Fever.				
Yes	370	194 (52.4)	0.52	0.470
Nausea				
Yes	243	138 (56.8)	1.82	0.178
Stomach discomfort				
Yes	343	193 (56.3)	2.62	0.106

DISCUSSION

Sero-prevalence is reported to vary with differences in geography, ethnicity, and the cut-off titre used[17] such that results from different studies are difficult to compare. However, our finding of a seroprevalence of 53.5% in children in the Central Region of Ghana was relatively higher than those reported in Orang Asli aborigines in Malaysia (29.3%)[18] and in Nigeria (30.4%[15] and 21.5%[14]) but lower than those reported in healthy adults in Indonesia (68.0%)[19] and Nepal (81.0%).[21]

In São Paulo, Brazil a sero-prevalence of 38.8% was reported in school children.[16] An ongoing study of the prevalence of helminth eggs in soil samples from different locations in Ghana reports that viable *Toxocara canis* eggs (7/151; 0.05%) were found in soil samples in public places (market and open places) and drains in flood prone areas posing a possible source of infection (Dr I Ayi, personal communication 2013). *Toxocara* eggs are prevalent in the environment resulting in high chances of ingestion through contamination and might explain the high seroprevalence recorded among the children in Central Region.

The test used TES-ELISA is a specific diagnostic test for toxocariasis with high specificity and shows no cross-reactivity with sera from individuals infected with *Ascaris lumbricoides*, hookworm, *Entamoeba coli*, or *Giardia lamblia*[18] or patients with trichinosis or ascariadis.[20]

The gender of the respondent did not seem to be a major factor related to infection with *T. canis* among the children in our study. Similar findings have been reported in Malaysia, Nigeria, and Spain. [18, 15, 22] On the other hand, there are reports of a predominance of *T. canis* infection in males explained by differences in the playing and social behaviours of boys which result in an increased exposure to *Toxocara* eggs.[23, 24, 25]

Our study reported differences in the seroprevalence among children on the basis of educational level. School-going children had significantly higher prevalence of *Toxocara* infection than the non-school going children. This may be due to exposure to *Toxocara* eggs in school compounds and parks because of easy accessibility by dogs after school hours.[16]

Among school-going children, seroprevalence increased from crèche to primary school but declined among those in the Junior High School, a trend similar to what was observed for seroprevalence on the basis of age of child (Figure 1).

The age of a child is correlated to his or her educational level: children at primary school are on average between 6 and 11 years of age while those at JHS are between 12-15 years of age. Seroprevalence was found to be higher in the former age group.

A significant difference was observed in *Toxocara* seroprevalence on the basis of health facility attended. A child was likely to attend a health facility within his or her district or metropolis. Children living in Cape Coast metropolis recorded a significantly lower seroprevalence than those from the other 3 sites (Table 2). A plausible explanation is that Cape Coast metropolis is relatively more urbanised with improved sanitary conditions compared to the more rural nature of the other places. Thus, children within Cape Coast may be less likely to be exposed to *T. canis* eggs in the environment. Similar findings of significantly lower seropositivity in children from urban environment compared to those from rural areas have been reported from Holland[26] and from Slovak.[27]

A significant difference was observed between pet (dogs and cats) ownership and infection with *T. canis* in our study, similar to findings by others.[9, 25] In Nova Scotia, dog ownership was found to be a significant risk factor for infection in rural children, whereas a household dog appeared to present no risk to urban children.[23] Children with pets in their homes may inadvertently acquire the infection through ingestion of eggs from their pets contaminating the immediate environment.

There was no significant difference between children who played with their pets and those who did not, a finding supported by others.[28, 29] On the other hand, the difference in seroprevalence in children with a habit of hand washing with soap before eating and those who do not was significant, supporting findings reported by Demirci and colleagues.[29] *T canis* infection among children in the Central Region may, therefore, be mainly through contaminated hands.

Although some children in this study reported of having symptoms such as body rashes, vomiting, weight loss, loss of appetite, fever, nausea and stomach discomfort these were not associated with sero-positivity (Table 4). It has been explained that symptoms depend on the organ affected and the magnitude of infection[5]. There are reports in literature that after ingestion of infective *Toxocara* eggs by paratenic host such as man, the larvae hatch in the stomach and migrate into the mucosa of the upper small intestine penetrating it and causing stomach discomfort, loss of appetite, nausea and vomiting.[30]

When large numbers of infective *Toxocara* eggs are ingested, more larvae migrate through the various tissues. The immune system, on detecting larval movement mounts strong attack against them resulting in symptoms such as body rashes, weight loss and fever.[31] Lower doses of *Toxocara* larva are insufficient to stimulate a protective immune response and may migrate continuously entering the eye causing ocular larva migrans (OLM) type of toxocariasis without evoking systemic signs and symptoms.[32]

Even though the difference in seroprevalence in those who reported coughing and those who did not was significant (p=0.017)[Table 4], it was not as highly significant as the difference between those who reported difficulty in seeing compared to those with no difficulty in seeing (n=0.005). The latter suggests that *Toxocara canis* infections among children in this study might be more of the ocular larva migrans type. This would need to be verified by further studies.

Study Limitations

Certain limitations were identified in this study. First, because of the limited time available, the study was conducted only on a small population of children in Central Region visiting any of four selected health facilities. Therefore, generalizing the results to the population of children in Central Region may not be warranted. In spite of this, efforts were made to use standard sample size calculation ensuring that statistically acceptable significant numbers were selected for the study.

Secondly, the cross sectional study design used only measures prevalence, and not incidence; therefore information on temporal sequence between the exposure and disease as well as prognosis and natural history of *Toxocara canis* infection among the study population could not be obtained in this study. Thirdly, this study was done to determine the exposure of study subjects to *Toxocara canis* by identification of specific IgG antibodies; thus the findings are on sero-prevalence and risk factors, and exclude clinical findings. To make up for this, structured questionnaire was designed to elicit information on toxocariasis-related symptoms experienced by the study subjects. To reduce recall bias the symptoms experienced were limited to six months or less prior to date of interview.

CONCLUSION

Sero-positivity was detected in more than half of the children recruited for the study. Exposure to *Toxocara* was more likely through ingestion of infective eggs from contaminated environment. Age and hospital visited but not gender seemed to be factors related to sero-positivity, even though there was significant difference in seroprevalence between male and female children in 11-15 years age group.

Risk factors associated with sero-positivity of *Toxocara* exposure among the children in the study included keeping of pets, playing with the soil and habit of not washing hands with soap before eating. The type of toxocariasis might be ocular larva migrans because of the strong association between sero-positivity and reported symptoms of difficulty in seeing clearly. This study has provided a stage for a broader population-based epidemiological survey especially among school children in Ghana.

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