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Seroprevalence of *Toxocara canis*-IgG antibodies in two rural Bolivian communities

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Abstract. A survey on *Toxocara canis*-IgG seroprevalence was carried out in two Bolivian communities (Mora and Zanja Honda) living in the Cordillera Province, Department of Santa Cruz. Two hundred and sixteen people, both males and females, 2 to 85 years old were sampled. Altogether, 73 people were positive (34%). The seroprevalence was 27% in Mora and 42% in Zanja Honda (p=0.022). No statistical correlations were found with sex and age. High prevalences were also found for intestinal helminths (hookworms, *Trichuris trichiura*, *Ascaris lumbricoides*, *Hymenolepis nana* and *Strongyloides stercoralis*). Positive association between *T. canis* seropositivity and presence of *T. trichiura* and between *T. trichiura* and hookworms were found. *T. canis* egg prevalence in dog population was found consistently higher in Zanja Honda than in Mora (40% *vs* 27%).

Key words: Bolivia, rural communities, toxocarosis, seroprevalence.

Human toxocarosis is probably one of the most widely spread zoonotic nematode infections, mainly in the areas where the relationship man-soil-dog is particularly close. Studies carried out in Latin America have underlined a large diffusion of the parasite (Shantz *et al.*, 1980; Magnaval *et al.*, 1994) and a close relationship between this zoonosis and socio-economic status of infected people. In some tropical areas of Venezuela, for example, seroprevalence ranged from 1.8% in urban citizens of medium-high socio-economic level to 20% in inhabitants of poor districts, and up to 25.6% in rural populations and 34.9% in Amazon Indians (Lynch *et al.*, 1988). Cammarota *et al.* (1989), in a study carried out in Buenos Aires (Argentina) on 60 children and 50 dogs, found a prevalence of 63% and 82%, respectively, and a seropositivity of 47.5% was observed by Agudelo *et al.* (1990) in Colombia. A more recent study by Lynch *et al.* (1993) on the seroprevalence of *Toxocara canis* in children (1 to 15-year-old) from a slum area of Caracas, Venezuela, showed that the infection was practically as common as that by *Ascaris lumbricoides* and concluded that toxocarosis represents a potential public health problem in tropical areas that is largely overlooked.

The aim of this study was to evaluate the seroprevalence of *T. canis* antibodies in two rural communities in an area of Bolivia where the socio-economic status is very low and people live in close relation with many stray dogs and are also highly exposed to other human parasites (Bartoloni *et al.*, 1993).

STUDY POPULATION

The study was carried out on 216 people from two rural communities, Mora and Zanja Honda, located in the Cordillera Province (Department of Santa Cruz) at an altitude of approximately 450 m.s.l. There are 544 inhabitants in Mora and 224 in Zanja Honda, and the principle activities are agriculture and animal breeding. The hygienic and sanitary conditions in both communities are very poor and the dog density is high (about 5 dogs per household).

Fieldwork was carried out during a study to evaluate intestinal parasites and iron status of the villages' children (Bartoloni *et al.*, 1995). A total of 216 people (89 males and 127 females) were sampled, 121 from Mora (51 males and 70 females) and 95 from Zanja Honda (38 males, 57 females). The overall age range was 2-85 years, young people being more abundant due to the fact that all children of both villages were tested whereas a random sampling of the remaining population was done. For both localities, subjects were grouped according to age: 2-6 years, 7-11 years, 12-16 years, 17-35 years and >35 years. Each subject underwent serological and coprologic examination to determine the presence of intestinal helminths which could interfere with serological results.

MATERIALS AND METHODS

Sera were analyzed by ELISA for specific anti-*T. canis* IgG using a commercial kit (*Toxocara* IgG specifiche, Lofarma Allergeni, Milano, Italy) working on

excretory-secretory antigens (TES-Ag) from second stage *T. canis* larvae (Brunello *et al.*, 1986). Sera to be assayed (in duplicate) were diluted 1:1,000, and the results were determined photometrically at 490 nm by a microplate reader (Titertek Multiskan Plus, Flow). On the basis of the ODs of known positive and negative controls, the cut-off was selected as 0.45. *Toxocara* seroprevalence was calculated according to sex, age and residence.

Coprologic tests for intestinal parasites were carried out by a modified ether-formalin concentration technique (Para-Pack Macro-Con System, Meridian Diagnostic, Inc, Cincinnati, OH, USA). The prevalence of each recovered species was calculated according to sex, age and residence.

Six sera from patients with positive fecal exams for *Ascaris lumbricoides* were examined to evaluate the specificity of the TES-ELISA. A crude antigen was obtained by Stomacher homogenation of *A. lumbricoides* adult worms (2 males and 2 females) in 5 ml of buffer phosphate. The homogenate was dialyzed with a tube of 3,500 dalton (SERVA Feinbiochemica) in 400 ml of buffer carbonate. Four ml of antigen with a proteic concentration of 3 mg/ml were obtained (proteic measurement of the homogenate performed according to Lowry *et al.*, 1951). A depletion with this antigen was carried out according to Underwood (1983): 20 ml of antigen were added to 50 ml of each sera and the solutions were maintained in slow agitation at 18°C for about 1 hour to allow antigen/antibody contact. The ELISA test was also performed on the same sera without depletion (50 ml of each sera plus 20 ml of buffer phosphate).

Fieldwork was completed by stool specimen collection from a total of 116 randomly selected dogs of both villages: 56 from Mora (19 puppies <1 year old and 37 elder dogs) and 60 from Zanja Honda (19 puppies <1 year old and and 41 elder dogs). Faeces were processed with the same method used for human stools and the presence of *T. canis* eggs was determined. The dog sample size was assessed on the basis of the dog population, according to the Epi-Info (Dean *et al.*, 1990) analysis.

The interactions between observed seroprevalences for *T. canis* and intestinal helminths, residence, sex, and age were assessed by log-linear models for the analysis of multi-way contingency tables. Single interactions between each factor were analyzed by partial association Chi-square. Predicted models were then fitted on observed data by Maximum Likelihood Chi square. Different *T. canis* egg prevalences in dogs from the two villages were analyzed by Pearson Chi square (Statsoft Inc Statistica package).

RESULTS

A total of 73/216 sera (34%) showed an ELISA reading over 0.45, with values ranging from 0.46 to 1.04. Positive people for anti-*T. canis* IgG were 32 males (36% of the males examined) and 41 females (32% of the females examined). The seroprevalence

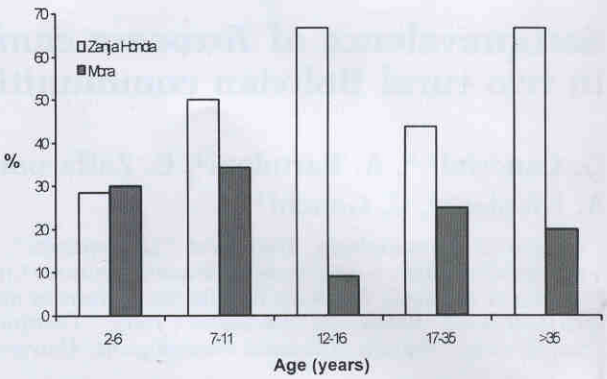

Fig. 1. Seroprevalence for *Toxocara canis* by age in the two villages.

was 27% (33/121) in Mora and 42% (40/95) in Zanja Honda (p=0.022). Figure 1 shows the seroprevalences of the different age groups in the two villages. Though the number of people in the >12-16-year-old classes are low/very low, the seroprevalence in this group tends to increase in Zanja Honda and to decrease in Mora. No differences related to age or sex were found in either village.

The prevalence of *T. canis* eggs in the dog population was 40% in Zanja Honda and 27% in Mora. The difference was not statistically significant. The positivity rates in puppies were 37% and 42%, in Zanja Honda and in Mora, respectively.

Coprologic examination showed 67 positive patients out of 121 (55%) in Mora: 27 males (53% of the males examined) and 40 females (57% of the females examined). Helminth prevalence was 50% (18/36) in the 2-6-year age group, 66% (25/38) in adolescents ranging from 7 to 11 years, 55% (6/11) in 12-16-year-old subjects, 52% (11/21) in the 17-35-year-old age group and 47% (7/15) in adults >35 years old. The most common helminths detected were hookworms (41%), followed by *Trichuris trichiura* (17%), *Hymenolepis nana* (11.5%), *Strongyloides stercoralis* (8%) and *A. lumbricoides* (7%). Tapeworm and pinworm eggs were also present (1%). Multiple infections were observed in 48.5% of positive samples: 41.1% had 2 worm species, 5.8% harboured 3 species and 1.4% had 4 species. No differences in overall fecal positivity and in each worm species prevalence were found between males and females and between classes of age.

In Zanja Honda, 63 patients out of 95 (66%) were positive to fecal exams: 22 males (58%) and 41 females (72%). The helminth prevalence was 67% (28/42) in 2-6-year-old children, 61% (14/23) in adolescents (7-11 years old), 100% (4/4) in the 12-16-year-old age group, 54% (7/13) in young adults ranging from 17-35 years and 77% (10/13) in adults >35 years old. Eggs of hookworms (56%), *T. trichiura* (26%), *H. nana* (13%), *A. lumbricoides* (12%), tapeworms (4%), pinworms (1%) and larvae of *S. stercoralis* (1%) were found. Overall, multiple infections were observed in 55.5% of positive

samples: 42.8% harboured 2 worm species, 11.1% had 3 species and 1.5% had 4 species.

Statistically significant higher seropositivity level for *T. canis* and higher prevalence of hookworms were detected in Zanja Honda. Positive associations between seropositivity and presence of *T. trichiura*, and between *T. trichiura* and hookworms were also detected. No association was found in either village between serological data and *A. lumbricoides* infections. Furthermore, the specificity of *Toxocara* ES antigens was confirmed by the analysis of 21 sera from patients coprologically positive for *A. lumbricoides* eggs: 15 sera out of 21 were ELISA negative and the other 6 ELISA-positive sera remained positive after depletion against a crude *A. lumbricoides* antigen.

DISCUSSION

An obvious bias of this study is the high number of children compared to the adult population and the over-representation of women, as indicated by the overall sex ratio of 0.7. However, toxocarosis appears to be widespread in the sampled population of these two rural Bolivian communities. The prevalence is significantly lower in Mora compared to Zanja Honda where a higher number of dogs, particularly of puppies, are positive for *T. canis*. Furthermore, the higher prevalence of geohelminthic infections in Zanja Honda suggests poorer living conditions in this village. The infection is spread homogeneously throughout the population and independently from sex and age.

It is worthy to note that 27% and 42% of the sampled population in the two villages respectively showed immune stimulation by *T. canis*. These values are higher than those observed by Lynch *et al.* (1988) in rural populations in Venezuela. Although the low number of adults studied in the two villages (27 subjects older than 35 years) did not allow to verify the cumulative effect of age observed in previous surveys (Thompson *et al.*, 1986; Genchi *et al.*, 1990; Magnaval *et al.*, 1994), our findings seem to confirm that in tropical areas *Toxocara* infection occurs throughout the lifespan (Magnaval *et al.*, 1994).

The high dog density, the observed prevalence of *T. canis* infection in these animals, the humid climate favouring the survival of parasite eggs in the soil, and the poor hygienic conditions (confirmed by the high prevalence of human soil-transmitted nematodes and mainly by the high presence of *H. nana*) may explain the high prevalence of *Toxocara* antibodies in these rural populations. Furthermore, the association between TES-antibody in sera and infections by some intestinal worms like *Trichuris* seems to confirm that soil contamination is the crucial factor for human infection.

No association was found between anti-*Toxocara* antibody prevalences and *A. lumbricoides* infection. Although it is not entirely correct to assume that sera of a patient having eggs of *A. lumbricoides* in stools should still be positive, and even though the depletion of the sera with somatic antigens does not necessarily neutralize anti-ES antibodies, the results of the analyses performed on the sera before and after *A. lumbricoides* antigen depletion seem to confirm the specificity of the TES-antigens used.

In agreement with studies carried out by other authors (Shantz *et al.*, 1980; Magnaval *et al.*, 1994) we observed high prevalences of *T. canis* antibodies that were not, however, associated with clinical symptoms of *larva migrans* syndrome. In fact, visceral *larva migrans* and ocular toxocarosis are practically unknown to local physicians. Further surveys to evaluate the actual incidence of vision impairments or other *Toxocara*-associated clinical features (e.g., epilepsy) are currently being planned in this area.

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