PROSPECTIVE SEROEPIDEMIOLOGICAL STUDY OF HUMAN TOXOCAROSIS IN BAHIA BLANCA, BUENOS AIRES PROVINCE, ARGENTINA

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ABSTRACT

The Inhabitants of Villa Nocito neighbourhood in Bahia Blanca, Buenos Aires Province, Argentina, suffers from many basic needs. In earlier studies, the authors have shown that 2.7% of dog feces collected in public places in the area had *Toxocara canis* eggs. In this paper, *T. canis* antibody prevalence is studied in sera from 94 local inhabitants of different ages and both sexes. Two ELISA tests were employed -LMD *Toxocara* Serology and a laboratory-made one. The Immuneblotting Technique (WB) was used as confirmatory test. Statistical analysis was performed with Fisher Test and EPIDAT 3.1, 2006 software.

Results showed 18% seroprevalence for *T. canis*. Mean sensitivity of ELISA tests was 96.79% with 86.55% of predictive value of positive result, without any significant differences between the two when compared with WB. No statistical significant risk associations were detected for sex, age, current water use, type of bathroom, contact with animals and earth, journeys, parasitosis history, and presence of special signs or symptomatology. Even though positive serology percentage was higher among those who had cesspit to dispose feces, this fact did not show a statistical significant difference (p: 0,14).

Results showed a lower seroprevalence for *T. canis* than other authors have reported in 1994 and are in agreement with the scarce presence of *T. canis* eggs in feces of local dogs.

INTRODUCTION

It is said that dogs are man's best friends but it is also true that they can transmit him diseases as they may have parasites. Although ascarides from dogs and cats, such as. *Toxocara canis* (Werner, 1782) Johnston, 1916 and *Toxocara cati* (Schrank**,** 1788) Brumpt**,** 1927, do not manage to continue or end their cycle in man, they do manage to infest humans and cause them disease in the erratic larval stages known as Visceral Larva Migrans (VLM) and Ocular Larva Migrans Ocular (OLM), both highly frequent worldwide. This makes their study an important step towards improving human health care.

Most puppies and kittens are born infested because they acquire this parasitosis transplacentally from their infested mothers.This pathology becomes relevant because of its high prevalence and the deposition of feces in public areas and recreational sites where children play. It should also be noted the children's frequent geophagic habit. *Toxocara* sp, eggs at the moment of being disposed are immature and need between 20 to 30 days to become infesting larvae (L2) in dogs, cats (varying according to breeds) and man.

Human infestation, generally in children, occurs when *Toxocara* sp larval eggs are ingested. Once in the digestive tract, the released L2 enter the blood stream and continue their cycle. If the host is a dog or a cat, they would normally end in the lungs, but if the host is a person, being an anomalous and accidental host, these larvae, which are very active, get lost and cannot continue their cycle thus wandering into different organs, mainly liver, brain and eye, where they reside extracellularly. In some cases, eosinophilic granulomas are formed as an inflammatory response to the parasites to kill the nematode, although they habitually remain viable and active for a year or more. The damage this larva causes is mainly mechanical and by inflammatory and immunological reaction often worsened by previous asymptomatic sensitivity.

Symptomatology will depend on the number of larvae present and on the affected organ. If larvae located in the eye, OML will be formed affecting the retina and will be more likely misdiagnosed as retinoblastoma. The lesion is habitually unilateral and may be accompanied with strabismus. VML is more frequent in children under 5 years of age. It manifests with fever, hepatomegaly and splenomegaly, respiratory signs and symptoms such as bronchospasms, almost 70% of eosinophilia, and hypergammaglobulinemia -types IgM and IgG-. Nephritis and myocarditis and involvement of the CNS have also been described. It is often asymptomatic, being eosinophilia the only detectable sign that leads to an immunological diagnosis. (Costamagna, 2008).

The diagnosis is habitually immunoserologic with the Enzyme-Linked Immunosorbent Assay (ELISA). An intradermal reaction with parasite antigen and indirect immunofluorescence are also employed.

Earlier studies demonstrated that 83.3% of the studied areas of the Napostá Stream that crosses Bahia Blanca City are polluted with eggs (Costamagna and col., 2002 y 2005), the infestation rate of *Toxocara* sp. found in feces of dogs from the city was 7% (Torno and col.

1996) and that in Bahia Blanca, with 300,000 inhabitants, there are more than 18,000 stray dogs and 50,000 owned dogs (Luciani, 1998).

The population of Villa Nocito Neighbourhood in Bahía Blanca suffers from unmet basic needs. Earlier studies have demonstrated that 87% of dogs feces found in that area had parasites of zoonotic importance. Even though *Ancylostoma* sp. was the most prevalent, *Toxocara sp* eggs were detected in 10.3% (33% in their larval stage) of the total positive samples. This meant a real prevalence of 2.7% (Baillie and col., 2007). Moreover, an 87% of the local children showed intestinal parasites (Costamagna y col, 2002).

The aim of this work was to study the seroprevalence for *Toxocara canis* prospectively in the population of Villa Nocito Neighbourhood in Bahia Blanca city from 2007 to 2008; and, secondly, to validate two ELISA methods for the detection of anti-*Toxocara canis* antibodies against Immunoblotting, and to relate them to the presence of *Toxocara sp* in dog´s feces from public places in the area.

MATERIALS AND METHODS

Blood samples were collected from 94 patients of all ages, 81 females and 13 males from Villa Nocito Neighbourhood (Bahia Blanca, Argentina) (Figs. 1 and 2) to determine antibodies type IgG for *T. canis.* Sociocultural and sanitary data were registered with the patients' consent.

Fig 1: Area under study, Villa Nocito

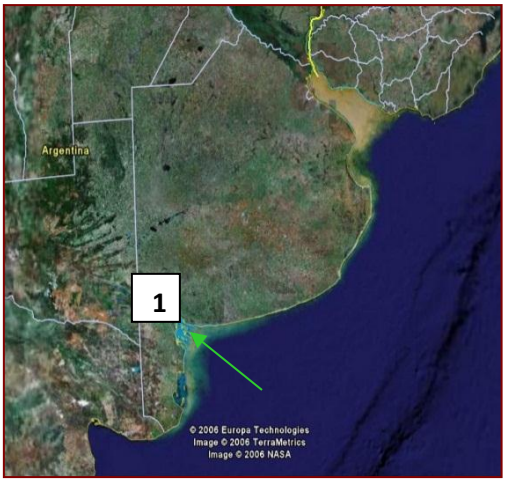

Fig 2: Bahía Blanca (arrow) Argentina. (arrow) Bahía Blanca, Argentina (**1**)

Two ELISA kits were employed, a commercial one: LMD Toxocara Serology Microwell ELISA, code No: 241000; and a laboratory-made one (ELISA Dr. Gabriela Echenique). As confirmatory test, the Immunoblotting technique (WB) was used. For the statistical analysis the Fisher test and EPIDAT 3.1, 2006 software were employed. .

House ELISA kit: Specific antibodies against the antigens of larval excretion/secretion L_2 of *T. canis* (TES) were determined in the patient's sera with qualitative sandwich enzyme immunoassay (ELISA) employing TES antigen

obtained according to Savigny technique. Dilution for sera was 1/64. Optic density reading (OD) was done at 490 nm in Dynatech Laboratories MRX plaque reader. Cut limit: 0.300. All sera were processed in triplicate.

Procedure:

12. **Coating** of cups with TES antigen.
13. **Washings:** cups were washed with a PBS buffer at pH: 7.4 and 0.1 % Tween 20.
14. **Blockage:** with PBS buffer, pH: 7.4 / lean milk 1.5 %, 1 hour at 37 ºC.
15. **Washings:** same as step 2.
16. **Dilution of patients' sera:** 1/64.
17. **Sera Seeding:** 50 µl serum (1/64) at 37 ºC, 90 minutes, in humid chamber. Two positive controls (weak and strong) and two negatives were also seeded.
18. **Washings:** same as step 2.
19. **Incubation with secondary antibody:** each serum was incubated with human anti-IgG (Sigma) marked with peroxidase at a concentration of 1/5000, 90 minutes at 37 ºC.
20. **Washings:** same as step 2.
21. **Sustrate addition:** 60 mM hydrogen peroxide in 50mM citrate buffer mM pH: 3.2 and 10mM tetramethylbenzidine (TMB) in chlorhydric acid 0.1 N, 30 minutes, at room temperature.
22. Reaction was obtained with H_2SO_4 2 N.
23. **OD Reading:** at 490 nm

Immunoblotting Technique (WB)

a) Protein electrophoresis in polyacrylamide gels in denaturalizing conditions: SDS-PAGE (10%) under experimental conditions according to the Laemmli method. TES proteins were transferred to nitrocellulose membranes and incubated for 1 hour with the patients´sera, dilution 1/64.

b) Protein Transference and Immunodetection in nitrocellulose membrane (Western Blotting): membranes were confronted for one hour with a protein conjugated with alkaline phosphatase. Human anti-IgG conjugated with alkaline phosphatase was employed.

The different antigen bands were detected with the color reaction of phosphatase employing 5-bromo-4-chloro-3-indol phosphatase (BCIP) with nitro blue tetrazolium (NBT).

RESULTS

Results showed an 18% seroprevalence for *T. canis*, in the studied area. Mean sensitivity of the ELISA kits employed (LMD *Toxocara* Serology and Dra. Gabriela Echenique's ELISA Test) was 96.79% with an 86.55% of Predictive Value of Positive Result, without significant differences between them against WB. When employing the range test with Wilcoxon sign, conclusion that both kits allow the determination of the same value (p= 0.856364) was reached-

No risk associations with statistical significance were found for the following: sex, age, current water use, type of bathroom, contact with animals and earth, journeys, history of parasitosis and presence of any special signs or symptomatology. Even though the positive serology percentage for *T. canis* was higher among those who used cesspit for excreta disposal, this difference was not statistically significant (*p:* 0.14). Association between positive serology for *T. canis* and journeys was observed (P: 0.04).

CONCLUSIONS

Human toxocarosis is the result of the prevalence of this parasitosis in dogs and the quantity of them that share the environment with man. Results in relation to the prevalence of *T.canis* in the dog's feces collected in public

places of the Villa Nocito neighbourhood in Bahía Blanca (Argentina) showed to be a 2.7% with 33.3% of larval eggs, i.e. infesting (Baillie and col., 2007) what corroborates the high resistance of eggs in the environment (Borchert, 1975) as weather conditions in the studied area are extreme, though dog's feces were collected in November 2006 with a temperature of about 12-16º C.

The *T. canis* seroprevalence in humans shown in this study by means of two ELISA test techniques and Immunoblotting (WB) was 18% and the two ELISA tests employed offered satisfactory results, with a good correlation between them and the WB.

Studies carried out in the pediatric population of Bahia Blanca in 1994 using the ELISA test, determined a 44.2% of immuneprevalence for Toxocarosis (Gentili and col., 1994). Two years later, Torno and col. demonstrated a 7% prevalence for *Toxocara* in feces of dogs in public places of the same city.

Other studies indicate that there is a 10% positivity among blood donors in the city of Gualeguaychú (Minvielle and col., 2000), a 39% in La Plata inhabitants (Radman, 2000) and 37.9% in children between 1 and 14 years of age in the city of Resistencia, Chaco (Alonso and col., 2004).

Although the results are within those found in other latitudes, education and awareness-raising actions have been started in the city to reduce seroprevalence rates for *Toxocara* in humans in the medium term. The aim of the actions also include reduction of: dogs' infestations and the quantity of feces from dogs and cats in public places and in sandboxes in child care centers and squares. These actions are performed by the University Volunteering of the chair: "*Erradicating parasites from child care centers and schools, with love, solidarity, and social and civic knowledge and responsibility*" (http://www.lasparasitosis.com.ar).

To conclude, it is considered relevant to quote part of the Technical Report 277 of WHO, 1964: "Helminths transmitted by soil" where it makes reference to the importance of different helminthosis from a Public Health point of view in its introduction, stating that: "…it can be said, without exaggerating, that parasitic worms undermine the health of human populations in almost all the regions in the world. Even in the most developed countries, where heminthoses are relatively benign or rare, the existing are accurately considered as harmful".

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