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Research Article

Toxocariasis in Two Ecosystems in Argentina

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Abstract

The purpose of this work was evaluate the situation of Toxocariasis in two ecosystems of the Argentina different in altitude, climate, environmental, demographic conditions, political situation, socio-cultural conditions and health system, such as the Department of Malargüe, Mendoza Province and the Department of Eldorado, Misiones Province.

The two departments have a significant population growth

1. Cuyo region, the selected area was the Department of Malargüe, the largest department, located at 1475 meters above sea level, with a population of 27660 inhabitants. The relief of the region has elevations over 3000 meters. Summer rainfall is scarce and the weather is warm and dry during this season.
2. The second selected area was the Department of Eldorado, in the Mesopotamian sub region, located in the northwest region of Misiones Province. This is an area of 1,927km with 78,152 inhabitants. This region has a tropical climate and no dry season. Northeast, Southeast and East are the prevailing winds. This biome consists in Iguazu tropical rainforest and gallery forest.

There are no adequate conditions of sanitation in the two departments in regards to water inside the house, and sewers. In Malargüe, found a 27.02% of dogs infected and 14.2% of samples with ascarids in the soil of house. Eldorado, stool samples showed a 61.9% of eggs of *Toxocara canis*. Finding a 64.2% of house contaminated with ascarids. In the two departments, 25% of the studied population had antibodies anti *Toxocara canis*. The presence of eggs in the soil of the two departments, show significant differences with 0.02755 confidence interval/0.57562 with 95% confidence. Finally we can conclude the findings of the present study show the zoonotic potential of this parasite, revealing the need to implement preventive health-care and educational measures, and to reassert the importance of Toxocariasis as a relevant public health problem.

Keywords: Diagnosis; Epidemiology; Toxocariasis; Surveillance

Introduction

Toxocariasis is a zoonotic infection caused by ingesting embryonated eggs of nematodes (Ascarids) that infect dogs and cats (*Toxocara canis, Toxocara cati*). Toxocariasis epidemiology has been intensively studied and it is now accepted that this zoonosis is primarily transmitted by contact with contaminated environments. Several studies based on the prevalence of *Toxocara spp.* In soil samples have been published worldwide [1-8]. Most of them were performed in the Northern Hemisphere, in urban or semi-urban environments with temperate, tropical and subtropical climates which favor the development of *Toxocara spp.* eggs. Among them, seven studies on seroprevalence were carried out in arid or semiarid climates (Canary Islands, Egypt, Greece, Northern India, Israel, Lebanon, and Southern Iran), where heat and drought can hinder the development of *Toxocara spp.* Eggs [9]. To date, studies performed in Argentina on Toxocariasis prevalence have been conducted on an irregular basis and used different methodologies and study populations. In La Plata city, 13% of public parks tested positive for *Toxocara spp.* [2], while 25.7% of *Toxocara spp* eggs were found in other cities such as Paraná, Santa Fe and Santo Tomé as well as in 53 out of the 68 (77.9%) areas of urban recreation [4]. In Resistencia city, Chaco Province, *Toxocara spp.* eggs were found in 33.3% of public squares, in 25.0% of public gardens, and in 20.6% of small squares. In 61.5% of the positive samples, eggs were embryonated or in the division process [1]. Another study performed on 2193 samples of dog feces in the Southern Greater Buenos Aires found 11% of *Toxocara canis* eggs [10]. In the city of Buenos Aires, 7.2% of the soils in public parks were contaminated with *Toxocara spp*. eggs [6]. Studies performed on cat feces collected from public institutions found 61.2% of positive samples with eggs of *Toxocara cati* [7].

A study conducted in three provinces of Argentine Patagonia revealed a high potential for environmental contamination with *Toxocara spp*. eggs (35.1%). However, the seroprevalence was 31.6%, suggesting that extreme weather of the Patagonian steppe inhibits the development of *Toxocara spp*. eggs [9].

This study was aimed to evaluate and compare the situation of Toxocariasis in two Argentine ecosystems at different altitudes, demographics, climatic and environmental conditions, as well as political, cultural and health systems: The Department of Malargüe in Mendoza Province, and the Department of Eldorado in Misiones Province.

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Materials and Methods

Regional geography

Within the Cuyo region of Argentina, the selected area was the Department of Malargüe, the largest department (41317 km^2) in Mendoza Province, located at 1475 meters above sea level, with a population of 27660 inhabitants [11]. The relief of the region has elevations over 3000 meters [11]. Summer rainfall is scarce and the weather is warm and dry during this season [12].

The second selected area was the Department of Eldorado, in the Mesopotamian sub region, located in the northwest region of Misiones Province. This is an area of 1,927 km with 78,152 inhabitants [13,14]. This region has a tropical climate and no dry season. Northeast, southeast and east are the prevailing winds. This biome consists in Iguazu tropical rainforest and gallery forest [15].

Secondary sources were investigated, such as the Argentina Population and Housing Census 2001, 2010, 2011 [13,16,17] and literature on climate and geographical situation of the two study regions [11,15,18]. Also, data on the health situation in the region of Cuyo and Northeastern Argentina (NEA) provided by the Ministry of Health of Argentina [19-22] and reports from the Secretariat of Agriculture, Livestock, Fisheries and Food [23] of the Center for the Implementation of Public Policies Promoting Equity and Growth (CIPPEC) [24] and the Directorate of Statistix and Economic Research from Cuyo (DEIE) [22,25] were also analyzed in order to know the regional aspects related to the demographics and socioeconomic dimensions as well as the organization of health services.

The health indicators selected were: Demographic indicators, include population pyramids and dependency ratios; environmental indicators, involve percentage of population with access to drinking water and sanitation, water resources and probability of natural hazards (flood, geological, geomorphological); socioeconomic and political indicators such as gross national product per capita, literacy rate, predominant economic activity, health expenditure, health system and population health insurance coverage.

Population-based Study

The observational unit was focused on families and their house. A simple random sampling was done. Twenty-one families were randomly selected from the two regions defined previously. Environmental samples were collected from available house and also samples from people who were present at that time. In the Department of Malargüe, families from the rural areas studied were made up of four people on average and ranged from 5 to 68 years old. In the Department of Eldorado, families were six people on average and ranged from 2 to 20 years old of both genres.

Blood samples were obtained by venipuncture after the subject or parents of the minors signed an informed consent. A structured form was completed containing basic demographic data such as age, gender, job position, ownership, animals, deworming of cats/dogs, type of anthelmintics used, diet, water supply and type of drainage and wastewater disposal. Serum samples were stored at -20°C until use. Research on human subjects was carried out in accordance with the provisions of the Universal Declaration of Human Rights of 1948, the ethical standards established by the Nuremberg Code in 1947, the Declaration of Helsinki of 1964 and subsequent amendments already regulated by the National Law 25.326 on data protection of individuals [26,27].

Collection of faecal samples from dogs

Thirty-seven canine faecal samples dispersed in the environment were collected in the Department of Malargüe and 10 canine faecal samples in the Department of Eldorado. More samples could not be obtained as ducks ate canine faeces. Samples were placed in wide-mouth plastic bottles and labeled according to the collection site: Dispersed in the environment, peridomiciliary area, dog pound, dog shed. Samples were stored at room temperature to be sent to the laboratory.

Collection of soil samples

In all, 18 and 21 soil samples were collected from house, peridomiciliary areas and dog sheds in the Departments of Malargüe and Eldorado respectively. Samples were collected by a superficial scraping of one square meter area previously delimited by a circle. Soil samples were placed in polyethylene bags and submitted to the laboratory. The volume of the soil sample was 300 g. per household. The total volume of soil or faecal samples was weighed, taking 10% of the total weight, and placed in a formol-saline solution for concentration parasitological methods at 1:2 weight-volume ratios.

Statistical Analysis

In the statistical treatment of data, the two-sample proportions test was applied using the statistix 8.0 software, setting the level of statistical significance in CI 95% with a P value of 5%. It was expressed through the null hypothesis of equality and the alternative hypothesis of a sample higher than the other.

Concentration techniques

Method with tween 80-citrate buffer: It was performed according to the procedure developed by Oshima et al. [28]. Samples were observed microscopically using 40x and 10x lenses.

Telemann method modified [29]: It was performed according to the procedure developed by Telemann, 1908. Samples were observed microscopically using 40x and 10x lenses.

Willis flotation method [30]: It was performed according to the technique developed by Willis, 1921. Samples were observed under a microscope using 40x and 10x lenses.

Sheather method [31]: It was performed according to the procedure developed by Sheather, 1923. Samples were observed under a microscope using 40x lenses.

Serological techniques

Western blotting: It was performed according to the procedure developed in the Department of Parasitology of the National Institute of Infectious Diseases "Carlos G. Malbrán" ANLIS [32]. Nitrocellulose membranes were blocked with PBS buffer pH 7.4/0.5% Tween 20, 5% skim milk, during 1h, and then washed 3 times for 5 minutes with PBS buffer pH 7.2/0.5% tween 20. They kept in the freezer at -20°C until use. Nitrocellulose strips incubated with sera diluted 1/100 in the buffer P/T/L (PBS pH 7.2/0.1% Tween 20/0, 5% skim milk) and incubated 1h with stirring at room temperature. The membranes

were washed as before, were incubated with anti-human IgG (Sigma: 8667) marked with peroxidase and diluted in the buffer P/T/L 1/1000, 1h at room temperature, with permanent agitation; the reaction was revealed with diaminobenzidine.

Total antigen ELISA assay: It was performed according to the technique described by Cerverizzo I et al. [33], with changes made in the Department of Parasitology of the INEI "Carlos G. Malbrán" ANLIS. In flat bottom inmunolon II 50µl of the antigen ES/L3 total, was coated, settled for 18 hours in the refrigerator. Washed 3 times with buffer (PBS pH 7.2/0.1% Tween 20) P/T for 5 minutes. Was blocked with PBS buffer pH 7.2/skim milk 1.5% for 1 h at 37°C. Incubated with 50µl of dilution of sera of patients with serum controls for 30 min at 37°C in a humid chamber. The wells were once again washed and incubated with 50µl of anti-human IgG (Sigma A 8667) marked with peroxidase, at a concentration of 1/5000, diluted with P/T. Washings were repeated and reaction is revealed by adding 100µl substrate acid 2, 2-Azino-bis (3-etilbenzotiazolin-6) sulfonic acid (ABTS), were incubated plates 10 min, the reaction stopped with 100µl of 0.1-N, pH 3.2 hydrofluoric acid, and was read at 410nm on a team Dynatech MR 4100.

Results

Indicators

According to the population of Malargüe, this is the 6th agglomerate of Mendoza Province. Its population pyramid is of a bell-type. In addition, this pyramid is called the progressive pyramid which means that it has a fast growing number of inhabitants. The Department of Eldorado in Misiones Province has a population pyramid with a high younger-age population with a wide base; the majority of its population is economically active [13].

In the Department of Malargüe, the Potential Dependency Ratio (PDR) is almost 55% similar to the whole country, but it is interesting to note that the PDR of the Department of Eldorado is 60% [3].

The environmental indicators are shown in table 1 [11,13,15].

Indicators	Malargüe	Eldorado
Population with access to drinking water	82%	77%
Population with access to sanitation	1.76%	4.3%
Water resources	Melt-water rivers	Large numbers of rivers
Probability of natural hazards (floods, geological and geomorphological hazards)	Volcanic eruptions	Floods

Table 1: Environmental indicators: Departments of Malargüe and Eldorado.

The only socioeconomic indicators assessed were: The gross domestic product per capita, literacy rate, and the prevailing economic activity. Results are shown in table 2 [13,18,22,24].

Political indicators, only the population health insurance coverage was available but data on the number of physicians per 1000 inhabitants in the Department of Eldorado was not available to compare the two departments (Table 2).

Indicators	Malargüe	Eldorado
Gross domestic product per capita	1,687 million pesos	4,617 million pesos
Literacy rate	4.4%	1.4%
Prevailing economic activity	Timber industry	Oil industry
Population health insurance coverage	38%	39%

Table 2: Socioeconomic, political and health indicators: Departments of Eldorado and Malargüe.

Immunoparasitological study

Table 3 shows the number of people studied in two populations, the families were randomly selected.

Department	Area	Families	Person (n)	Western Blot (+)	%
Malargue	Rural	21	20	5/20	25
Eldorado	Rural	21	40	10/40	25
Total (N)		42	60		

Table 3: Number of people studied in both departments.

The 25% samples analyzed by serology in the two departments presented antibodies anti Toxocara spp. Results from soil samples and dog faeces are shown in table 4. The number of faeces samples in Eldorado is lower because were eating for ducks.

Department	Samples with Ascarids Eggs in Soil	Samples with Toxocara Eggs in Faeces	% of Dogs with Toxocara Eggs	% of Ascarids Eggs in Soil
Malargüe	3/21	10/37	27.02	14.2
Eldorado	8/18	4/10	61 9	64.2

Table 4: Prevalence of Toxocara eggs in environmental samples from the Departments of Malargüe and Eldorado.

Table 5 shows the relationship between the percentage of positive serology by Western blot and dogs found to be Toxocara carriers in both Departments.

Dogs Found to be Toxocara				
	Malargüe		Eldorado	
	Yes	No	Yes	No
Positive	15%	0%	17.5%	10%
Negative	10 %	75 %	7.5%	65%

Table 5: Relationship between the percentage of positive serology and dogs found to be Toxocara carriers in the Departments of Malargüe and Eldorado.

Discussion

Toxocariasis is little known by pet owners who lack ideas on ways to take necessary measures to minimize infection risks. The main factor for contracting this parasitosis is the presence of animals parasitized with *Toxocara spp.* in the environment, a situation that causes soil contamination with parasite eggs [34,35]. Several studies conducted worldwide have shown increased rates of soil contamination in urban settings such as parks, public gardens, recreation areas,

squares and sandpits [33,34]. However, studies in the United States and Southwestern France showed that people living in rural regions had a higher risk of becoming infected with Toxocara eggs than those living in urban areas [4,6].

In this work two different ecosystems such as the Department of Malargüe (Mendoza) and Eldorado (Misiones) with uneven sociocultural and environmental conditions were evaluated. A significant population growth was observed in both departments which was demonstrated by the high Potential Dependency Ratio (PDR) [13] leading to an increase in the canine population, 25,000 dogs in Malargüe, and therefore increasing the risk of contracting this parasitosis.

The sanitation level is another parameter of additional exposure that may interact with the infectious power of contaminated soils as stated elsewhere [2,3,10,34,35]. In this study the health system is inadequate in both departments, although the Department of Malargüe is in better conditions since 78.9% of the population uses motor pumps.

It could also be observed that both departments have seriously a very poor sewer connection. In the Department of Malargüe, only 1.76% of the population has a sewer network and 4.3% in the Department of Eldorado. It is very striking that more than half of the population in Malargüe, regardless of gender, has no health coverage at all [18,22].

It can be observed that the NEA is the Argentine region that has allocated less money to the health sector regarding its public spending, representing 11.1% of the 10,843 million pesos spent in 2006 [21]. Of note, in the Department of Malargüe, besides being pets, dogs perform rural activities and therefore defecate elsewhere, spreading parasites in a wider territory that can be favored by western winds blowing at a constant speed, which is related to the results found in this study, since despite we found 27.02% of infected dogs, only 14.2% of ascarid samples were found in houses.

Most of the houses studied in Eldorado had more than one dog and the faecal samples represented 61.9% of *Toxocara canis* eggs. Note worthy, these houses had no fences, and as a result, dogs wandered along public spaces contaminating the entire surrounding area with *Toxocara canis* eggs accounting for 64.2% of house contaminated with ascarids, even the floors of the houses with no dogs.

Likewise, the habit of walking barefoot in Eldorado and playing on the floor, besides the poor management of excreta, makes it the optimal scenario for the transmission of Toxocariasis. However, despite the differences in climate, social factors related to health care, state of housing, sanitary conditions and habits and behavior of the population, the serological result from both populations measured by WB have a similar outcome (Table 3). There might be an ecosystem in balance; diseases do not represent a danger but a factor of "natural selection". Human activities and alterations of the environment have created new dynamics and new patterns for infectious diseases that favour the spread of pathogens, both geographical and between species [36].

Regarding the percentage of soil and faecal samples positive for *Toxocara spp*. (Table 4), a greater rate can be observed in the Department of Eldorado. This could have been influenced by the time of year when the sampling was conducted besides the weather condition, favorable for the parasite that needs moisture and room temperature, a suitable substrate for the larvae to develop, considering that *Toxocara spp*. eggs need to spend time on the floor to be able to infect humans. Misiones Province, as described before, presents a bioenvironmental diversity favorable for maturation and retention of Toxocariasis in humans and animals. The results found in this study are in line with those found in other subtropical areas [3,37,38].

The value of infestation found in this study is close to that reported for the city of Santa Fe (25%), located in Santa Fe Province [10]. Regarding the relationship between positive serology and its association with keeping dogs in the two regions studied (Table 5) there are no significant differences in their percentages as to conclude that animal keeping is a determining factor for the human population to acquire this parasitosis. (The two-sample proportions test, using statistix 8.0, showed no significant difference in CI 95%; limits were -0.32225/0.38151).

This study showed that the presence of *Toxocara spp*. was more frequent on the floor of houses in the Department of Eldorado than in Malargüe (64.2% vs. 14.2%). The two-sample proportions test using statistix 8.0 revealed that the presence of eggs in the soil of the two Departments present significant differences, CI 95% (0.02755 / 0.57562).

This demonstrates the importance of the common environment and the interaction with contaminated areas and the persistence of parasite eggs as an impact factor to consider in future decision-making activities involving the health-care system.

We can conclude that the geographic distribution of *Toxocara spp*. depends largely on weather conditions, soil types and the behavior of susceptible populations. The findings of the present study show the zoonotic potential of this parasite, revealing the need to implement preventive health-care and educational measures, and to reassert the importance of Toxocariasis as a relevant public health problem.

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