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A SEROLOGIC STUDY OF HUMAN TOXOCARIASIS IN THE CANARY ISLANDS (SPAIN): ENVIRONMENTAL INFLUENCES

J. F. JIMENEZ, B. VALLADARES, J. M. FERNANDEZ-PALACIOS, F. DE ARMAS, AND A. DEL CASTILLO
Department of Parasitology and Department of Ecology, University of La Laguna, La Laguna, Tenerife, Spain

Abstract. The presence of anti-*Toxocara* antibodies in the human population of the Canarian Archipelago was studied by means of an indirect enzyme-linked immunosorbent assay with excretory/secretory antigen of infective-stage larva. A total of 14,074 were analyzed, resulting in the following distribution and prevalence by location: southern Tenerife 6,435 (2.5% positive), northern Tenerife 1,710 (6.7% positive), La Palma 1,214 (4.5% positive), La Gomera 264 (6.8% positive), El Hierro 204 (3.9% positive), Gran Canaria 2,875 (2.3% positive), Fuerteventura 277 (1.8% positive), and Lanzarote 1,095 (2.9% positive). The weighted average of the entire Archipelago was 3.4% positive. Neither age nor sex was found to be significantly associated with positive serology. The influence of the different Canarian mesoclimates on the spreading of this zoonosis is demonstrated, with significant differences being found between humid and arid islands. Likewise, a significant positive correlation was found between De Martonne's humidity-aridity index and percentage of people with evidence of parasite exposure on the different islands.

Toxocariasis is a zoonosis produced by the ascarids of cats and dogs, the main representative of which is *Toxocara canis*. In most cases, the illness develops discreetly. Visceral larva migrans (VLM) and ocular larva migrans are the two most important syndromes associated with the parasite in humans.[1,2]

Humans can be infected by ingesting parasite eggs or by contact with larvae contaminating the teats of bitches that have recently given birth, or the muzzle of the puppies, or by means of the paratenic hosts of the parasite.

The typical profile of patients with VLM was outlined by Glickman and others[3] and Schantz and others[4] in a five-year-old child with PICA habits and recently exposed to dogs. Subsequently, Magnaval and others[5] and Glickman and others[6] extended the profile to include other populations at risk: hunters, dog owners, etc. Likewise, Bentley and others[7] found a greater index of infection in veterinary surgeons and dog handlers. On the other hand, Lynch and others[8] demonstrated the influence of the socioeconomic conditions of the area on the index of infection, with differences ranging from 1.8% in areas with a high socioeconomic level to an incidence between 10% and 30% in marginal neighborhoods of Caracas, Venezuela. Lynch and others found appreciable differences in seropositivity according to the age of the population surveyed.[9] To enable the parasitic eggs to mature into infective larva, conditions of high humidity, high temperature, and sunlight are necessary because these are determinant factors for the development of the larvae.[10]

The objective of the present study was to analyze the presence and prevalence of antibodies against *T. canis* in the inhabitants of the Canary Islands, taking into consideration that the antibodies persist in the blood for at least five years postinfection,[11] and to establish the influence of the different Canarian mesoclimates on the spreading of this zoonosis.

MATERIALS AND METHODS

Samples. A total of 14,074 human sera from the Canarian Archipelago were studied (Table 1). Samples were taken during two consecutive years from the outpatient departments of eight different hospitals and health centers belonging to the National Network of Public Health throughout the Canary Islands. Place of residence, sex, and age of each patient were recorded. Public health patient cards did not provide information on occupation. Samples were divided in two age classes: 1) children ($\leq$ 12 years of age), when the serum was obtained from the pediatrics sections of the hospitals, and 2) adults ($>$ 12 years of age), when this was not the case.

Antigen. A secretory-excretory antigen taken from infective larva of the parasite and obtained by the method described by De Savigny and others[12,13] was used. This culture and diagnostic method is considered to be the most suitable by the majority of researchers.[14–16]

Enzyme-linked immunosorbent assay (ELISA). An indirect ELISA was carried out on microtiter plates for the detection of antibodies in sera with an antigenic concentration of 10 μg of protein/ml. The conjugate was anti-immunoglobulin G (IgG) (heavy and light chain) (Miles, Inc., Elkhart, IN) labeled with peroxidase at a dilution of 1:1,500. In each well, development was carried out with 100 μl of a solution containing 25 ml of citrate buffer (pH 5.1), 75 μl of 9% (w/v) hydrogen peroxide, and 16 mM *o*-phenylenediamine. The reaction was stopped with 2N sulfuric acid and the optical density (OD) values were read at 420 nm. Sera whose OD values were equal to or higher than the average value plus four standard deviations[17,18] were considered positive.

Climatic data. Climatic data on the Canary Islands were obtained from the zonal center of the Instituto Nacional de Meteorologia located in Santa Cruz de Tenerife.

Statistical analysis. To find significant seropositivity differences among age, sex, and locality of residence, we used a chi-square test ($P = 0.01$) and the Student's *t*-test ($P = 0.01$), respectively. Correlations among De Martonne climatic indexes and the seropositvity percentage of each island was calculated using the Bravais-Pearson correlation coefficient.

RESULTS

Results showed no significant association (χ^2 test) between age or sex (Table 1) and infection with toxocariasis. The existence of significant differences ($P < 0.01$, by the Student's *t*-test) in the seropositivity percentage between the western islands (5.8%) (including the northern slopes of Te-

TABLE 1

Epidemiologic analysis of toxocariasis seropositivity in the Canary Islands*

	No. of analyzed samples	No. positive	% positive	Significance
Age classes				
Children (≤12 years)	1,397	56	4.0	
Adults (>12 years)	12,677	399	3.1	Nonsignificant
Sex				
Males	6,523	229	3.5	Nonsignificant
Females	7,551	226	2.9	
Residence				
Eastern islands			2.5	
Tenerife (South)	6,435	165	2.5	
Gran Canaria	2,875	66	2.3	
Fuerteventura	277	5	1.8	
Lanzarote	1,095	32	2.9	Significant
Western islands			5.8	$P < 0.01$†
Tenerife (North)	1,710	116	6.7	
La Palma	1,214	55	4.5	
La Gomera	264	18	6.8	
El Hierro	204	8	3.9	
Total	14,074	455	3.2	

* The number of samples analyzed, positive number, percentage of the total number, and significance level of a χ^2 association test for different age and sex classes, as well as of Student's *t*-test differences in seropositivity percentages between two groups of localities (western and eastern islands) are given.
† Eastern versus western islands.

nerife, as well as La Palma, La Gomera, and El Hierro) and the eastern islands (2.5%) (including the southern slopes of Tenerife, as well as Gran Canaria, Fuerteventura, and Lanzarote) was confirmed (Table 1). To correlate the climate with infection frequency, the De Martonne humidity-aridity indices (annual precipitation in mm/annual mean temperature in °C = 10),[19] were calculated for each island capital, with the exception of Tenerife-North, where an average between the localities where samples had been collected, La Laguna and Puerto de la Cruz, was considered (Table 2). Percentages of seropositivity and climatic indexes were thus

TABLE 2

Climatic data of the Canary Islands including mean annual temperature, annual precipitation (mm), and the De Martonne aridity-humidity indexes (P/T + 10)*

Island	Annual mean precipitation (mm)	Annual mean temperature (°C)	De Martonne index
Tenerife (South)	243.5	20.8	7.90
Gran Canaria	140.0	20.2	4.63
Fuerteventura	105.0	18.8	3.64
Lanzarote	146.0	20.2	4.83
Tenerife (North)	505.0	17.2	18.57
La Palma	513.8	20.3	16.95
La Gomera	496.6	20.9	19.58
El Hierro	408.7	18.8	14.18

* P = precipitation; T = temperature.

subjected to a regression analysis and the results are shown in Figure 1.

DISCUSSION

Differences found in seropositivity rates between the western and eastern islands have been interpreted as a consequence of the more humid climate of the higher western islands, when compared with the arid climate of the eastern ones. Tenerife is the largest island and occupies a central position in the archipelago. With elevations to 3,718 meters and prevailing northeast winds, the island is divided into two well-differentiated sectors: 1) a north-windward slope that is humid and similar to the climate of the western islands, and 2) a south-leeward slope that is dry and similar to the climate of the eastern islands.[20]

A climatic index relating these parameters, such as the De Martonne index, should be, if our assumptions are correct, a good predictor of the frequency of this zoonosis on the different islands. Indeed, we found the percentage of infection on each island (Table 1), and even within a single island, as is the case of Tenerife, to be significantly correlated with

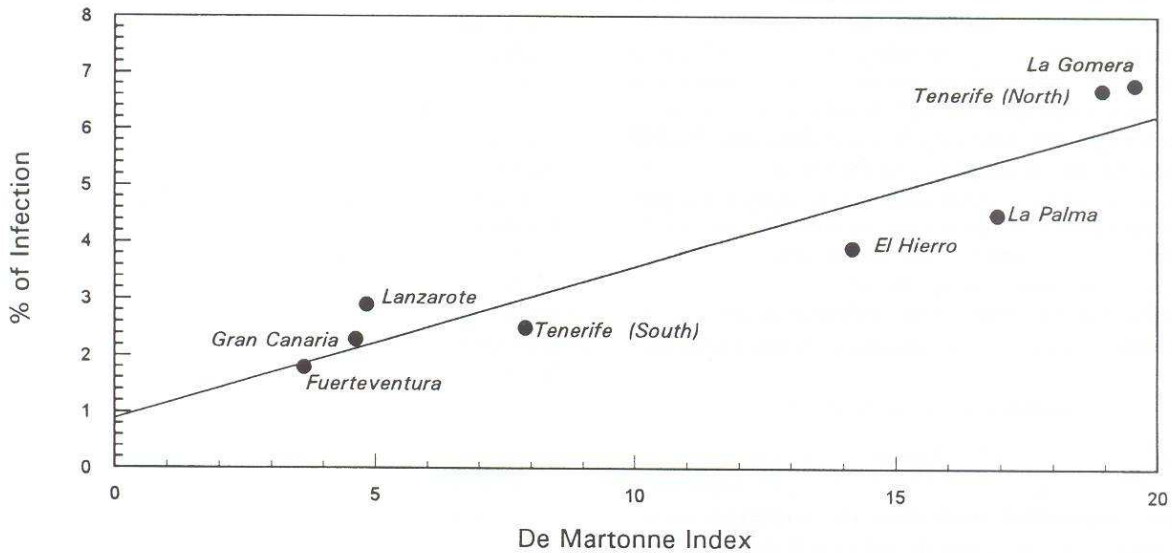

FIGURE 1. Regression of percentages of infection on each island and their De Martonne indices. A significant positive correlation ($r = +0.899$ and $P < 0.01$) was found.

its De Martonne index ($r = + 0.899$ and $P < 0.01$) (Figure 1). Nonetheless, the existence of a residual level of zoonosis on very arid islands such as Fuerteventura or Lanzarote (Table 1) provides evidence of the presence of alternative modes of infection that are not controlled by climatic features. These include paratenic host infection or the direct infection from female dogs and pups.[21]

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Authors' addresses: J. F. Jimenez, B. Valladares, F. De Armas, and A. Del Castillo, Department of Parasitology, University of La Laguna, Avda. Astrofisico F. Sanchez, s/n, 38207 La Laguna, Tenerife, Spain. J. M. Fernandez-Palacios, Department of Ecology, University of La Laguna, Avda. Astrofisico F. Sanchez, sn, 38207 La Laguna, Tenerife, Spain.

REFERENCES

1. Beaver PC, 1956. Larva migrans. *Exp Parasitol 5:* 587–621.
2. Glickman LT, Grieve RB, Lauria SS, Jones DL, 1985. Serodiagnosis of ocular toxocariasis. A comparison of two antigens. *J Clin Pathol 38:* 103–107.
3. Glickman LT, Chaudry IU, Costantin J, Clack FD, Cypess RH, Winslow L, 1981. PICA patterns, toxocariasis, and elevated blood lead in children. *Am J Trop Med Hyg 30:* 77–80.
4. Schantz PM, Glickman LT, 1978. *Toxocara* visceral larva migrans. *N Engl J Med 298:* 436–439.
5. Magnaval JF, Marchesseau P, Larrouy G, 1983. Les syndromes de larva migrans visceral ascaridienne dans la region Midi-Pyrinees. *Bull Soc Pathol Exot Filiales 76:* 69–75.
6. Glickman LT, Magnaval JF, Domanski LM, Shofer FS, Lauria SS, Gottstein B, Brochier B, 1987. Visceral larva migrans in French adults: a new disease syndrome? *Am J Epidemiol 125:* 1019–1934.
7. Bentley R, Daly M, Harris T, 1980. *Toxocara* infection among veterinarians. *Vet Rec 106:* 277–278.
8. Lynch NR, Wilkes LK, Hodgen AN, Turner KJ, 1988. Specificity of *Toxocara* ELISA in tropical populations. *Parasite Immunol 10:* 323–337.
9. Lynch NR, Hagel I, Vargas V, 1993. Comparable seropositivity for ascariasis and toxocariasis in tropical slum children. *Parasitol Res 79:* 547–550.
10. Cuellar C, Fenoy S, Aguila C, Guillen-Llera JL, 1986. New data on the photodependence of *Toxocara canis* ova for embryonation. *Rev Iber Parasitol 46:* 409–417.
11. Fenoy S, Aguila C, Cuellar C, Gillen-Llera JL, 1992. Persistence of immune response in human toxocariasis as measured by ELISA. *Int J Parasitol 22:* 1037–1038.
12. De Savigny DH, 1975. In vitro maintenance of *Toxocara canis* larvae and simple method for the production of *Toxocara* E/S antigen for use in serodiagnostic test for visceral larva migrans. *J Parasitol 61:* 781–782.
13. De Savigny DH, Voller A, Woodruff AW, 1979. Toxocariasis: serological diagnosis by enzyme immunoassay. *J Clin Pathol 32:* 284–288.
14. Van Knapen, Van Leusden J, Buys J, 1982. Serodiagnosis of toxocaral larva migrans by enzyme-linked immunosorbent assay (ELISA) with somatic adult and secretory larval antigens. *J Parasitol 68:* 951–952.
15. Abo-Sehada MN, 1992. Seroprevalence of *Toxocara canis* antibodies in humans in northern Jordan. *J Helminthol 66:* 75–78.
16. Bowman DD, Mika Grieve M, Grieve RB, 1987. *Toxocara canis:* monoclonal antibodies to larval excretory/secretory antigens that bind with genus and species specificity to the cuticular surface of infective larvae. *Exp Parasitol 64:* 458–465.
17. Portus M, Riera C, Prats G, 1989. A serological survey of toxocariasis in patients and healthy donors in Barcelona (Spain). *Eur J Epidemiol 5:* 224–227.
18. Riera C, Portus M, 1988. Diagnistico de la toxocariasis humana mediante ELISA con antigeno excretor/secretor de larvas 2 de *Toxocara canis. Rev Iber Parasitol 48:* 85–100.
19. Marzol V, 1984. El Clima. Afonso L, ed. *Geografia de Canarias.* Volume I. *Geografia Fisica.* Santa Cruz de Tenerife: Editorial Interinsular, 157–202.
20. Fernandez-Palacios JM, 1993. Climatic responses of plant species on Tenerife, the Canary Islands. *J Veg Sci 3:* 595–602.
21. Glickman LT, Schantz PM, 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol Rev 3:* 230–250.