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FACTORS ASSOCIATED WITH CONTAMINATION OF PUBLIC PARKS (HUÁNUCO, PERÚ) BY *TOXOCARA CANIS* EGGS AND OTHER ENDOPARASITES OF ZOONOTIC IMPORTANCE

FACTORES ASOCIADOS A LA CONTAMINACIÓN DE PARQUES PÚBLICOS (HUÁNUCO, PERÚ) CON HUEVOS DE *TOXOCARA CANIS* Y OTROS ENDOPARASITOS DE IMPORTANCIA ZOONOTICA

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Abstract

The objective of this work was to evaluate the contamination of public parks in the district of Huánuco, Perú with eggs of *Toxocara canis*. 32 soil samples were taken in each of 11 parks for a total of 352 samples in May and June of 2014. Parks were selected at random. In each park, 2-3 kg soil was collected according to the double "W" method. Flotation with a saturated sugar solution was used. Samples were considered positive when at least one egg of *T. canis* was found. Of the 11 parks sampled, *T. canis* eggs were found in 90.9%. Seven parasites were found along with *T. canis* in the soil. The sequence of parasite prevalence was in the following descending order: *T. canis* > Strongylus type eggs of *Ancylostoma caninum* > *A. caninum* larvae = *Blastocystis hominis* > *Trichuris vulpis* = *Entamoeba* sp. > *Capillaria* sp. = *Balantidium coli*. Parasite richness was 2.45 ± 1.21 (1-4) parasites per public park. A significant positive correlation was observed between the area of public parks and parasite richness per public park. No association was found between the condition of the public park and it being positive for *T. canis*. A dendrogram of similarity of association of parasites present in the 11 public parks showed a greater association between the *Strongylus* egg type and larvae of *A. caninum*. Also, a greater similarity existed between *T. vulpis*, *B. coli*, *Entamoeba* sp. and *Capillaria* sp. *T. canis* had the lowest similarity with the other parasitic forms. An increase in the prevalence of *T. canis* was observed in comparison to the previous decade as well as to the presence of other infective parasite stages of public health importance. People and especially children could become contaminated while in public parks and sanitary measures should be taken to control this zoonosis.

Key words: *Ancylostoma* - contamination by eggs - parasite - public parks - *Toxocara*.

Resumen

El objetivo de este trabajo fue evaluar la contaminación de parques públicos del distrito de Huánuco, Perú por huevos de *Toxocara canis*. En cada uno de los 11 parques fueron tomadas 32 muestras por lo que se evaluaron un total de 352 muestras de tierra en mayo y junio del 2014. La selección de los parques fue al azar. De cada parque se recolectó 2 a 3 kg de tierra, según el método de la doble "W". Se usó el método de flotación con solución saturada de azúcar. Se consideró positivo cuando se encontró al menos un huevo de *T. canis* por parque evaluado. De los 11 parques muestreados se encontró huevos de *T. canis* en el 90,9%. Siete fueron las formas parasitarias acompañantes a *T. canis* en el suelo. La secuencia de prevalencia parasitaria presentó el siguiente orden descendente: *T. canis* > huevos tipo *Strongylus* de *Ancylostoma caninum* > larvas de *A. caninum* = *Blastocystis hominis* > *Trichuris vulpis* = *Entamoeba* sp. > *Capillaria* sp. = *Balantidium coli*. La riqueza parasitaria fue de 2,45±1,21 (1 a 4) parásitos·parque público^{-1}. Se observó una correlación positiva significativa entre el área del parque público y la riqueza de parásitos·parque público^{-1}. No se encontró asociación entre el estado de conservación del parque público y la positividad con *T. canis*. Un dendrograma de similitud de asociación de los parásitos presentes en los 11 parques públicos mostró una mayor asociación entre huevos tipo *Strongylus* y larvas de *A. caninum*. También una mayor similitud entre *T. vulpis*, *B. coli*, *Entamoeba* sp. y *Capillaria* sp. *T. canis* presentó la menor similitud con el resto de formas parasitarias. Se observó un aumento en la prevalencia de *T. canis* en comparación a la década anterior, así como la presencia de otros estadios infectivos de parásitos de importancia en salud pública. Las personas y especialmente los niños pudieran contaminarse durante su estadía en los parques públicos y deben tomarse medidas sanitarias para el control de esta zoonosis.

Palabras clave: *Ancylostoma* - contaminación por huevos - parásito - parques públicos – *Toxocara*.

INTRODUCTION

Soils contaminated by eggs of helminth parasites of animals may constitute a zoonotic risk for humans and affect millions of people (Romero-Núñez *et al.*, 2011; Iannacone *et al.*, 2012; Marques *et al.*, 2012; Traversa *et al.*, 2014). There have been many different parasitic helminths with dogs (*Canis lupus familiaris* Linnaeus, 1758) as definitive hosts which can be transmitted to humans causing parasitic disease (Rojas 2003).

Toxocariasis is a geohelminthic, mainly asyptomatic, zoonotic disease caused by *Toxocara canis* (Werner, 1782) and *Toxocara cati* (Schrank, 1788) (Romero-Núñez *et al.*, 2011; Khazan *et al.*, 2012; Trejo *et al.*, 2012; Maragui *et al.*, 2014). Puppies with intestinal infections with *Toxocara* can excrete eggs of this helminth into the environment (Martínez-Barbadosa *et al.*, 2008; Maragui *et al.*, 2014). These eggs remain in the soil until larval development in 6 weeks, increasing the possibility of infection to humans and causing a risk to public health (Huapaya *et al.*, 2009; Armstrong *et al.*, 2011; Trejo *et al.*, 2012). *Toxocara* larvae can migrate to the liver, lungs, muscles and brain (Marques *et al.*, 2012) and can cause the syndromes of visceral larval migrans (VLM) and ocular larval migrans (OLM) (Martínez-Barbadosa *et al.*, 2008; Bartosik & Rzymowska, 2010; Marques *et al.*, 2012; Maragui *et al.*, 2014).

Transmission of toxocariasis is mainly through fecal matter disseminated in the environment (Armstrong *et al.*, 2011; Marques *et al.*, 2012; Maragui *et al.*, 2014), to which humans have free access through contact with the soils of parks and gardens frequented by canids (Santarém *et al.*, 2012). The presence of environmental contamination with *Toxocara* eggs is considered to be the best indicator of infection risk to humans (Marques *et al*., 2012; Santarém *et al*., 2012; Saraei *et al.*, 2012; Maragui *et al.*, 2014).

The hookworms (*Ancylostoma* spp.) and *Trichuris vulpis* (Froelich, 1789) are among the most important helminth parasites in dogs in environmental and clinical terms. Several studies have demonstrated the relevance of these two helminths in parks, green areas and beaches (Bartosik & Rzymowska, 2010; Iannacone *et al.*, 2012; Traversa *et al.*, 2014.).

High numbers of irresponsible dog owners, such as owners who do not pick up the feces of their pets, can lead to contamination by a large quantity of fecal material disseminated in recreation and leisure areas (Trejo *et al*., 2012). Therefore, the aim of this study was to address the following research question: What is the state of soil contamination by eggs of *T. canis* in public parks in the district of Huánuco, Perú?

MATERIALS AND METHODS

Area and type of study

The study was descriptive, transversal and comparative. Eleven parks in the district of Huánuco, Huánuco, Perú (09°55'58?S; 76°14'30?W) were sampled (Table 1). Parks were selected randomly. The average size of the parks was 985.09 ± 240.69 m^2. 352 soil samples were evaluated between May (n = 192) and June (n = 160) of 2015, with 32 samples taken from each of the 11 parks.

Soil samples

From each park was collected 2-3 kg of soil, according to the double "W" method, which consists of drawing in the sample area two "W", superimposed and inverted, with the length of each "W" determined in steps and the earth collected on each side of the "W". Each sample was taken at a depth of approximately 2.5 cm and a diameter of 5 cm (Guerrero, 1975). The samples were labeled with the name of the park and placed in polyethylene bags, stored at room temperature and taken to the laboratory for parasitological analysis corresponding to the environment (Zibaei *et al.*, 2010; Armstrong *et al*., 2011; Matesco *et al*., 2011; Khazan *et al.*, 2012). The method of Parodi Alcaraz was used (concentration method by flotation without centrifugation, using a supersaturated Benbrook sugar solution) (Daprato *et al*., 2011; Maragui *et al.*, 2014). Soil samples were placed in a mortar and the supersaturated sugar solution added and mixed well, using the pestle to crush coarse pieces. The obtained mixture was filtered through a funnel with a metal filter into 15 ml test tubes; the tubes were filled to form a meniscus and then covered with a glass coverslip. After 20 min the coverslip was removed and the slide examined under a microscope. Identification of *Toxocara* was made according to their morphology and typical size (Saraei *et al*., 2012). Samples were considered positive when at least one egg of *Toxocara* per sample was found (Harbinder *et al.*, 1997). Classification of eggs according to their state of development, e.g., single cell, pre-embryonated, and embryonated was not made. Viability of eggs was also not evaluated (Daprato *et al*., 2011; Romero-Núñez *et al.*, 2011). Similar parasitic forms present in each sample (helminth eggs and larvae, protozoan cysts) were analyzed according to specific identification guides.

Condition of public parks

Public parks were classified in the following manner: (1) Well preserved: parks entirely covered (100%) by grass; (2) Moderately preserved: parks approximately 50% covered by grass; and (3) Poorly preserved: neglected parks entirely without grass and with earth and sand only (Huaraca, 2001).

Data analysis

Spearman's correlation (r_s) between the area and the condition of each public park was performed and the parasite richness for each park examined. The Chi-square statistic also was used to associate park condition and prevalence of *T. canis*. A dendrogram of similarity of association (cluster analysis) of parasites found in the public parks of Huánuco, Peru was performed based on the algorithm of group pairs, using Ward's method with the respective correlation coefficients.

RESULTS

Table 1 shows the presence of *T. canis* and other forms of parasitic helminths and protozoans in 11 public parks in Huánuco. The sequence of parasite prevalence presented the following descending order: *T. canis* > *Strongylus* type eggs of *A. caninum* > hookworm larvae (*A. caninum*) = *Blastocystis hominis* (Swayne & Britton, 1849) Brumpt, 1912 > *T. vulpis* = *Entamoeba* sp. > *Capillaria* sp. = *Balantidium coli* Malmsten, 1857 (Table 1). The parasite richness was 2.45 ± 1.21 (1-4) parasites per public park. The sequence of the parasite richness of public parks provided the following descending order: 23 de febrero = Plaza mayor = Amarilis > San Pedro (Ramón Castilla) = Puelles > San Cristobal = San Sebastian = San Francisco > Santo Domingo = Heroes Huanuqueños = San Pedro (Infantil de tránsito) (Table 1). A significant positive correlation between the area of the public park and the parasite richness per public park (r_s = 0.74; p = 0.009) was observed.

Of the 11 parks, 3 were well preserved, 7 were moderately preserved and 1 was in poor condition (Table 2). The well preserved and poorly preserved public parks showed 100% positivity and the moderately preserved showed only 85.71% (Table 2). No association was found between the condition of the park and the positivity with *T. canis* (X^2 = 2.29; p = 0.32). Likewise, no correlation between park condition and parasite richness was seen (r_s = 0.43; p = 0.19).

Figure 1 shows a dendrogram of similarity of association among the parasites co-occurring with *T. canis* in the 11 public parks of Huánuco (correlation coefficient of cluster analysis = 0.87). A greater association between *Strongylus* type eggs of *A. caninum* and larvae of *A. caninum* was seen. Also, there was a greater similarity between *T. vulpis* and *B. coli*, and between *Entamoeba* sp. and *Capillaria* sp. *T. canis* had the lowest similarity with the rest of the parasitic forms present in the public parks based on the method of Ward.

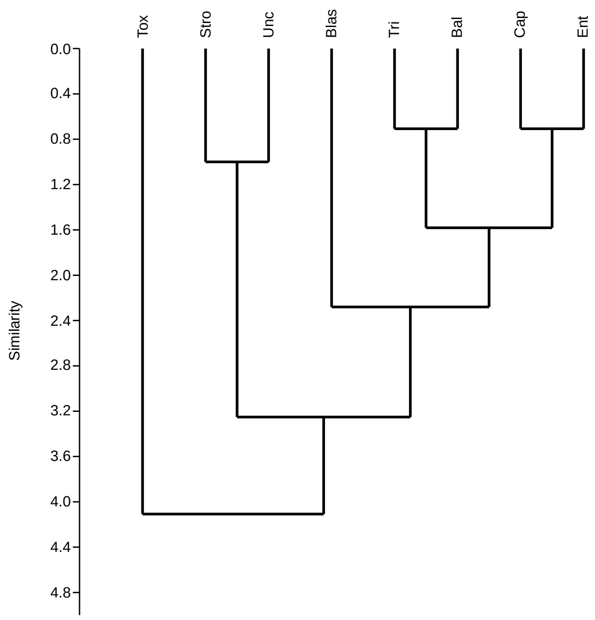

Figure 1. Dendrogram of cluster analysis similarity of parasites found in public parks in Huanuco, Peru based on the algorithm of group pairs according to Ward's method (r = 0.87). Tox = *Toxocara canis*. Stro = *Strongylus* egg type of *Ancylostoma caninum*. Tri = *Trichuris vulpis*. Cap = *Capillaria* sp. Unc = larva of *A. caninum*. Blas = *Blastocystis hominis*. Ent = *Entamoeba* sp. Bal = *Balantidium* coli.

Table 1. Surface area, condition and presence of parasites in 11 public parks of the district of Huánuco, Huánuco, Perú. *Tox* = *Toxocara canis*. *Stro* = *Strongylus* egg type of *Ancylostoma caninum*. *Tri* = *Trichuris vulpis*. *Cap* = *Capillaria sp*. *Unc* = larva of *A. caninum*. *Blas* = *Blastocystis hominis*. *Ent* = *Entamoeba sp*. *Bal* = *Balantidium coli*. EC = Condition. 1 = Well preserved. 2 = Moderately preserved. 3 = Poorly preserved. % MP = percentage of samples positive for *T. canis*. + = Presence of the parasite.

Public Park	área m^2	EC	*Tox*	*Stro*	*Tri*	*Cap*	Unc	*Blas*	*Ent*	*Bal*	Richness	% MP
Plaza mayor	1100	1	+		+				+	+	4	37.5
Santo Domingo	970	2	+								1	50
San Cristóbal	650	1	+					+			2	34.7
San Sebastián	580	2	+	+							2	78.13
San Francisco (Cartagena)	980	2	+	+							2	53.13
Amarilis	1020	2	+	+			+	+			4	31.25
San Pedro (Ramón Castilla)	1000	2	+	+			+				3	62.50
San Pedro (Infantil de transito)	980	3	+								1	53.13
23 de febrero (tramo puente San Sebastián - Cementerio)	1450	2		+		+	+		+		4	0
Héroes Huanuqueños (Tabaco)	876	2	+								1	56.25
Puelles (Los periodistas)	1230	1	+		+			+			3	12 50
Prevalence (%)			90.90	45.45	18.18	9.09	27.27	27.27	18.18	9.09		

Table 2. Number and frequency of positive parks – contaminated with *Toxocara canis* according to their condition in the district of Huánuco, Huánuco, Perú.

Condition	# of parks	(%)	Positive	(%)
Well preserved	3	27.27	3	100
Moderately preserved	7	63.63	6	85.71
Poorly preserved	1	9.09	1	100
Total	11	100	10	90.90

X^2 = 2.29, P =0.32.

DISCUSSION

Several investigations have determined the soil contamination of public parks in different socioeconomic levels worldwide (Khazan *et al.*, 2012; Iannacone *et al.*, 2012; Saraei *et al.*, 2012; Sudhakar *et al*., 2013). The contamination by eggs of *T. canis* (90.9%) is very high in the district of Huánuco, Huánuco, Peru and could cause infection in people who use public parks and recreational áreas (Khazan *et al.*, 2012; Sudhakar *et al*., 2013).

The identification of *T. canis* eggs in the soil of public parks of Huánuco, Perú is a bioindicator of the presence of infected dogs that defecate in these areas of rest and relaxation, and a high source of contamination to people (Saraei *et al.*, 2012; Trejo *et al.*, 2012; Sudhakar *et al*., 2013). Children engaged in recreational activities in these parks are at a higher vulnerability and epidemiological risk caused by this helminth (Khazan *et al.*, 2012; Sudhakar *et al*., 2013).

A positive correlation was observed between the area of the public park and the parasite richness per public park. Zibaei *et al.* (2010) found that parks more than 10000 m^2 were contaminated more than those of smaller size. Conversely, Trejo *et al.* (2012) in México City found that the size of green area had no influence on the presence and viability of eggs of *Toxocara sp.*

A high association between *Strongylus* type of eggs of *A. caninum* and larvae of *A. caninum* was seen. Traversa *et al.* (2014) indicated that *Ancylostoma* spp. is, after *Toxocara*, the species most prevalent in parks and gardens with an average prevalence in 7 investigations in America of 39.02%. The high association found between the eggs and larvae of *A. caninum* in the parks of Huánuco is evidence of continuous contamination with this geohelminth.

With respect to the condition of the public parks, 6 parks were moderately preserved, 3 parks were well preserved and one park poorly preserved; these were contaminated with *T. canis* eggs, accounting for 85.71% (6/7), 100% (3/3) and 100% (1/1), respectively. This means that there was no relation between the presence of *T. canis* eggs and the condition of the parks. Our results are in contrast to the information found by Huaraca (2001), who noted that the moderately preserved, well preserved, and poorly preserved parks were contaminated at 100% (2/2); 77.7% (7/9) and 0% (0/1), respectively, possibly due to the preference of canids to defecate in areas more carefully constructed. Soils that retain moisture are better for the developmental cycle of the eggs of *T. canis* in contrast with the parks that had only soil and cement (Marques *et al*., 2012). In addition, Pomé *et al.* (2013) noted that the risk factors for contamination by *Toxocara* are: public lighting, no use of trash containers, presence of green areas, dog-walking without a leash and outside sale of prepared foods.

Several environmental and sociocultural factors may influence the presence of *Toxocara* in soils of public parks such as: weather, soil texture, season, latitude, altitude, sunshine, geographic location, procedure of soil collection, sampling depth, number and volume of samples, and size of the park, among others (Zibaei *et al.*, 2010;

Daprato *et al.*, 2011; Matesco *et al.*, 2011; Santarém *et al.*, 2012; Sudhakar *et al.*, 2013).

Inadequate official sanitary measures in relation to responsible pet ownership that avoids or reduces the presence of dog feces in public parks could explain the high levels of contamination with *Toxocara* (Khazan *et al.*, 2012; Santarém *et al.*, 2012). In the territory of Peru, there is the Supreme Decree No. 006-2002-SA with its Rule of Law No. 27596 that regulates responsible ownership of dogs, and the municipal ordinance No. O4:2014-MPH-LL that standardizes the ownership, protection and control of dogs in the district of Huánuco, Huánuco, Perú. In particular, in this last regulation the owners are obliged not to permit their dogs to roam or leave their homes and should properly remove their feces or droppings. Our results show that the regulations regarding responsable dog ownership are not being fulfilled efficiently in the district of Huánuco, Huánuco, Perú.

The percentage contamination with *T. canis* eggs (90.9%) in this study showed an increase in the last decade in relation to that previously recorded in the same locality (Rafael, 2000 cited by Iannacone *et al.*, 2012; Pujay, 2000 cited by Iannacone *et al.*, 2012; Huaraca, 2001). This could be due to the different times of year in which samples were collected, apparently being favorable in the present case in which the rainy season was extended until May, while previous surveys were conducted mainly during the dry season (Daprato *et al.*, 2011; Santarém *et al.*, 2012). Iannacone *et al.* (2012), in an analysis of the prevalence of eggs of *Toxocara* sp. in parks and recreational areas in several Latin Americans countries, found a lower prevalence in Peruvian Andean localities of high altitude (Cusco, 32.6% at 3399 masl and Puno, 25% at 3827 masl) in comparison with Huánuco (90.9% at 1894 masl).

Among the helminths reported in this study, *A. caninum* has been reported in Peru in dogs and domestic cats, as well as in the fox *Lycalopex culpaeus* (Molina, 1782) in several locations such as Cajamarca; Cusco; Huancavelica; Chincha (Ica), Huancayo (Junin); Huarochiri, Lima, Tayacaja, Yauyos (Lima); Iquitos, Maynas (Loreto); Tahuamanu (Madre de Dios); and Puno (Puno) (Sarmiento *et al*. (1999). Prevalences have been seen up to 12% in Lima and 52% in the Mantaro Valley (Leguia, 2002), and its importance in public health has been recognized with the clinical symptoms known as cutaneous larval migrans (Leguía, 2002).

Trichuris vulpis has been reported in the departments of Cusco and Lima (Sarmiento *et al.*, 1999), with a 10% prevalence found in Lima (Leguía, 2002). Their eggs are very resistant to adverse environmental conditions and remain viable for years (Leguía, 2002) while their zoonotic potential is still under discussion (Traversa, 2011). *Capillaria* sp. has been reported in several species of birds and domestic and wild mammals in several provinces of Peru (Sarmiento *et al*., 1999); in the case of *Calodium hepaticum* (Bancroft, 1893) Moravec, 1982 (syn. *Capillaria hepatica*), various mammals, including mice and rats as well as some carnivores such as dogs and cats, are involved in their life cycle (Taylor *et al*., 2007), with their zoonotic potential recognized in our area (Tantalean, 1994).

Among the protozoa *B. coli* has been reported in pigs in Lima and Callao (Zaldivar, 1991) and in other countries in various domestic and wild mammals, including dogs and cats (Taylor *et al*., 2007), besides being reported in several clinical cases in the Peruvian population (Pamo *et al*., 1999). *Blastocystis hominis* has been reported in a wide variety of animals (Taylor *et al*., 2007) including domestic dogs (Wang *et al*., 2013), proving its zoonotic potential and having reported human cases in our country (Salinas &Vildazola Gonzales, 2007). Finally, *Entamoeba* sp. also has been reported from a wide variety of animals; the species most important in public health, *E. histolytica* Schaudinn, 1903, has been reported sporadically in domestic animals like dogs (Taylor *et al*., 2007) and is present in the human popuation of Perú (Cornejo *et al*., 1999).

Several of these parasites have not been reported previously contaminating parks of Peru and their presence could be a bioindicator that dogs and cats, other domestic animals (such as rodents and pigs), wild animals (e.g., wild pigeons, possibly indicated by the presence of *Capillaria* sp.) and even the likely presence of human feces could explain our findings in the public parks of the city of Huánuco.

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