

Contamination of Beach Sand by Cutaneous Larva Migrans in Rabat-Sale Area (Morocco) and their Health-Related Problems

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ABSTRACT

Environmental contamination by soil-transmitted helminths is a public health problem worldwide and has been well documented by several authors. Visceral *larva migrans* and cutaneous *larva migrans* are two examples of these soil-transmitted helminths caused by *Ankylostoma*. Unfortunately, limited studies have described the presence of such contamination in the saline soils of coastal beaches. Thus, the current study was carried out on the beaches of the Rabat-Sale region (Morocco) in the period between June and August 2014 to determine contamination by *Ankylostoma* larvae. 100 sand samples were taken and analyzed using the flotation technique. The frequency of the *Ankylostom* spp. in samples from public areas was 33%. At all collection sites, the presence of feces from stray dogs was observed. Generally, it is important to adopt measures to control breeding dogs, treat infected animals and provide health education for the population.

INTRODUCTION

Geohelminths are parasites that require soil to complete a stage in their development cycle, allowing eggs to develop into viable embryos and larvae until contact with a new host. The infectious forms of these soil-transmitted helminths (eggs or larvae) are most often found in sandy soil, contaminated with human or animal feces (Acuña *et al.*, 2003). According to the OPAS 2002 report (Ehrenberg, 2002), it is estimated that two billion people in the world are infected with a form of parasite acquired through contact with the ground; 800 million children are infected (40%), with about 20 to 30% in Latin America (Rey, 2001).

The presence of mammalian feces in urban soil is a significant public health problem in several countries, mainly due to the presence of parasites that can cause larva migrans syndrome in humans. There are traditionally recognized zoonotic diseases, such as

visceral larva migrans caused by *Toxocara* spp. and cutaneous larva migrans caused by *Ancylostoma* spp. These parasitic zoonoses are associated with the presence of animals, mainly dogs and cats, in places where humans can be infected, such as squares and public parks (**Despommier, 2003; Hotez & Wilkins, 2009; Rybinsk-Elefant *et al.*, 2010; Lotfi *et al.*, 2020**).

The increasing number of domestic animals, particularly in large urban centers, has increased contact between animals and humans and escalated the risk of increased exposure to parasites responsible for zoonosis. The invasion of the human body by nematode parasites from other animals is an atypical development of the parasitic species involved, which is unable to complete its life cycle. Under these conditions, it is obvious that the infectious larval form cannot pass to the adult worm, and its abnormal migration in the host stops in various tissues such as the skin, eyes, liver, lungs among others. Nematodes that penetrate through the skin, but still wander between the epidermis and the dermis, causing clinical cutaneous larva migrans (**Hotez & Wilkins, 2009**).

The populations of dogs and cats that circulate in the streets can access public places of leisure and, during defecation, contaminate the ground with several types of parasites resulting from zoonotic transmission. This contamination has become a critical public health problem, particularly in the tropical and subtropical regions of the developing countries.

Sandy soils represent a main source of human infection by parasites due to their geological characteristics, being formed by sand particles with diameters ranging from 0.02 to 2mm, with the ability to retain water between the spaces of soil particles. The larval stages of helminths are particularly aquatic, and high soil moisture is essential for their survival. In addition, the pattern of rain, the rate of evaporation and the amount of sun to which the contaminated soil is exposed, strongly influence both the development of eggs in embryos and the viable larval stages. Ideal conditions for the rapid development of the parasite are provided by frequent rain all year round in places with sandy soils, which are not directly exposed to the sun and are protected from intense evaporation. The optimum temperatures for the development of the parasite in the soil varies between 23 and 30°C, with an upper limit of 40°C at the upper end; bottom development becomes slower at temperatures below 17°C and stops below 10°C (**Rybinsk-Elefant *et al.*, 2010**).

In accordance to the afore- mentioned data, the aim of this work was to assess the frequency of cutaneous larva migrans in the sand of the beaches of the Rabat-Sale region, Morocco.

MATERIALS AND METHODS

Sampling and stool

In 10 different beaches, 10 samples of 500g of sand were taken from a depth of 10cm in the period between June and August 2014. The areas studied are located in the areas where most children play. The samples were packed and transported in plastic bags to the Parasitology Laboratory of the National Institute of Hygiene in Rabat, where they were analyzed by the spontaneous sedimentation method of the Lutz, Hoffman, Pons and Janer.

Sand samples were washed using tap water and subjected to centrifugation at 2,500 revolutions/ 3 min. After the removal of the supernatant, samples of the sediments were obtained from sand at the bottom of the sedimentation tube using a micropipette, then transferred to a glass slide and subjected to microscopic control of the parasitic structures.

The sediment remaining in the bottom of the sedimentation was treated using the flotation technique. After removing the supernatant and resuspending the top two centimeters of sand deposited at the bottom of the sedimentation, it was subjected to a brief centrifugation. The deposit formed in the bottom of the centrifuge tube was diluted in a solution of zinc sulphate ($d= 1.180$).

The identification of the larvae causing larva migrans *Ancylostoma* spp. was performed based on morphology using an optical microscope.

The fresh faces collected in the morning, in clean plastic, are immediately stored in a 10% formalin solution and transported to laboratory of the National Institute of Hygiene Rabat.

RESULTS

With 100 sand samples collected, 33% (33/100) contained *Ankylostoma* larvae. For the frequencies of contamination of beaches' sand under study, it was determined that, the Des Nations beach is the most contaminated beach, with 70% positive samples, followed by the Sale (50%), Casino (40%), Sable beach. d'Or, Amphitrite, Rose Marie and Kasba (each represented 30%); while, the Conterbandier and Sidi Abed recorded frequencies of 20% and 10%, respectively (Table 1).

Table 3. Frequencies of *Ankylostoma* larvae in the beach sand of the Rabat Sale region

Beach	Number	Positive effect	Frequency(%)
Plage des Nations	10	7	70,00
Sale	10	5	50,00
Oudaya	10	2	20,00
Sables d'Or	10	3	30,00
Cazino	10	4	40,00
Contrebandier	10	2	20,00
Sidi Abed	10	1	10,00
Amphitrite	10	3	30,00
Rose Marie	10	3	30,00
Kasba	10	3	30,00
Total	100	33	33,00

Variation by month was detected in the frequencies of the parasites. The highest contaminated sand samples was recorded in August with 39.3%, followed by July (33.3) and June (27,2) (Table 2). Moreover, the beach of Sidi Abed was only contaminated in August; no larvae of *Ankylostma* spp. were detected in June and July. Statistical analysis showed a significant difference ($p < 0.05$) (Fig. 1). In the beaches studied, the presence of stray dogs was noted. In addition, we detected the presence of dog feces dispersed across several places in the sand of the beaches.

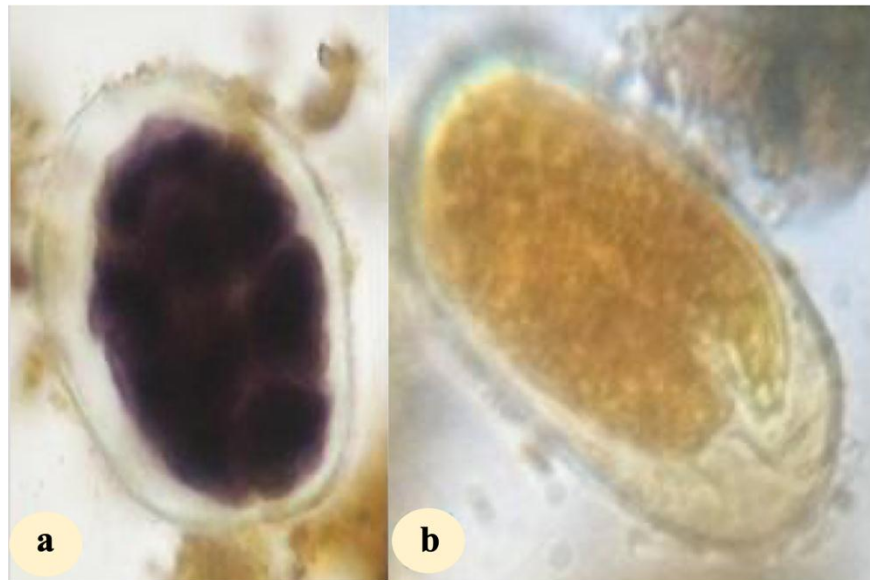


Fig. 1. *Ankylostoma spp* (a) and its larva (b).

Table 2. Univariate analysis of the frequency of skin larva migans founded in the beach sand of the Rabat Sale Region.

Month	Juin		July		August		Statistical analysis (ddl=3)		
	N	(%)	N	(%)	N	(%)	khi-2	p	IC (95%)
Plage des Nations	1	14,29	2	28,57	4	57,14	1,83 0,6	0,07	1,06
Sale	2	40,00	1	20,00	2	40,00	3,14 0,37	0,07	1,06
Oudaya	1	50,00	1	50,00	0	-	4,64 0,2	-0,2	0,49
Sables d'Or	0	-	1	33,33	2	66,67	3,19 0,36	0,16	0,73
Cazino	1	25,00	2	50,00	1	25,00	2,85 0,41	0,06	0,92
Contrebandier	0	-	2	100,00	0	-	1,07 0,78	0,16	0,73
Sidi Abed	0	-	0	-	1	100,00	10 0,01	0,16	0,73
Amphitrite	0	-	0	-	3	100,00	3,19 0,36	0,16	0,73
Rose Marie	2	66,67	1	33,33	0	-	3,19 0,36	0,16	0,73
Kasba	2	66,67	1	33,33	0	-	3,19 0,36	0,16	0,73
Total	9	27,27	11	33,33	13	39,39			

DISCUSSION

The soils of public squares and parks are the main route of parasitic zoonoses to the human transmission population. Among these zoonoses are cutaneous larva migrans caused by *Ancylostoma spp* (Heukelbach and Feldmeier, 2008; Lotfi *et al.*, 2020). Studies on the prevalence of parasites causing *larva migrans* have been carried out in various regions of the world, including either examinations of dog and cat stools, or assessments of soil contamination for helminth eggs and larvae (Mizgajska, 2001; Lee *et al.*, 2010; Carvalho and Rocha, 2011). In general, many authors have sought to assess the degree of soil contamination in public places by measuring *Toxocara spp.* and are less interested in contamination of the environment by eggs or larvae of *Ancylostoma spp.* (de Castro *et al.*, 2005).

The larvae of *Ancylostoma spp.* have been found in soil samples worldwide. They are present in public and private places such as playgrounds, parks, sand pits, sidewalks, streets, gardens and fields. In this study, we found that the soil studied is mainly sandy, and more rarely clay soil. Sand soils have been described as an important source of human infection by parasites (Rocha, 2011).

In the present study, we have a fairly high frequency (33%) of the sand samples analyzed are positive by the larvae of *Ancylostoma spp.* in the 10 beaches of the Rabat-Sale region. This frequency is high compared to that of other cities in certain countries, such as Bogotá, Colombia (10.7%) (Polo-Terán *et al.*, 2007), Kaduna, Nigeria (9%) (Maikai *et al.*, 2008), Wrocław, in Poland (4.9%) (Perec-Matysiak *et al.*, 2008), Madrid, Spain (3%) (Guimarães *et al.*, 2005) and different regions of Costa Rica (2%) (Dado *et al.*, 2012). With regard to Brazilian cities, our results are lower than those recorded in Araçatuba (State of São Paulo) by Nunes *et al.* (2000) who found 46% of sand samples contaminated with larvae of *Ankylostoma spp.*

On the other hand, the degree of *Ankylostom larva* contamination was higher than recovered in certain cities, such as Fernandópolis, São Paulo (1.8%) (Nunes *et al.*, 2000), Santos (15%) (Cassenote *et al.*, 2011), Other cities like Duque de Caxias and Araçatuba, São Paulo. the frequencies found are respectively (33.5%) (Thomé *et al.*, 2008), (37.5%) (Heukelbach *et al.*, 2008) In contrast, the higher contamination rates than our results were noted in Santa Maria (Rio Grande do Sul) by Corrêa & Moreira (1996), who found 93.3% of the land public places with eggs contaminated with *Ancylostoma spp.* In addition, Guimarães *et al.* (2005) reported the presence of hookworm eggs and larvae in 69.6% of soil samples from public places in the city of Lavras (Minas Gerais).

The results obtained in this study indicate that the majority of the municipal population is at zoonotic risk. In fact, the contamination of beach sands can be explained by the

presence of animals which can release eggs or larvae and parasites into the environment through their excrement and thus generate a risk for the population. The high prevalence in other areas indicates that socio-economic indicators, such as low income and education, are linked to the spread of parasites with zoonotic potential in the environment (**Sommerfelt *et al.*, 2006**). In addition, the density of the canine population varies from region to region, especially stray dogs.

In our study, it was noted that many animals (residence, domicile or semi-stray) defecated in the sands of the beaches studied. These dogs therefore represent an important aspect in the transmission of zoonoses in urban areas. In addition, from an epidemiological point of view, the World Health Organization (WHO) recommends that the dog population in each location should not exceed 10% of the human population (**WHO, 1990**).

We have noticed that the parasites are frequently found in sand in contact with water suggesting that the parasites are sensitive to high concentrations of salt. On the other hand, **Silva and Pastura (2000)** showed in a study that there is absence of structure of parasites in the sand taken near water located between the pavement and the mark of the high tide.

However, on the beaches studied, we found that there is a wide variety of recreational activities, such as sports fields and socializing areas are concentrated, in addition to a large number of garbage. Also, there is a large concentration of people between 11am and 2pm or at the end of the day, and where people stay for long periods of time.

On the other hand, we found that the analyzed samples containing large amounts of viable parasitic structures that occur in all months of collection. This can be explained the chemical and climatic factors that influence their viability in the environment for long periods of time, the type of soil, ambient temperature and humidity are the main factors that determine the time it takes to evolve from egg to larvae (**Mizgajska, 2001**). In the Rabat-Sale region, between June and August 2014, the summer season, the average temperature was 30 ° C and therefore it has the right temperature to promote the maintenance and development of nematode or larvae eggs.

The evaluation of the contamination of sand by parasites of zoonotic relevance carried out on the beaches of Corrientes, in Argentina, by **Milano & Oscherov in 2002**, revealed a positive rate of 32.7%, with a prevalence of 100% for the larvae of Ankylostome. parasite contamination was considered to be present when at least one positive sample was found for the parasites mentioned above. These data are in agreement with our study on the beaches of the region of Rabat. In contrast, the study carried out by **Laggagio *et al.* (2001)** on three beaches in the municipality of Guaiba, Rio Grande do Sul again showed a different parasite profile once again, with a low prevalence of hookworms.

A study by **Cáceres *et al.* (2005)** on the southern beach of Ilhéus, Bahia, evaluated the presence of nematode larvae in sandy soil at different depths, and showed the presence of *Strongyloides stercoralis* (41.66%) and larvae of Ankylostomes (58, 33%) without significant differences related to the depth of the sample taken, which is explained by the behavior of rhabditoides and filarioides larvae which exhibits positive and negative geotropism, respectively.

The results presented here are a major cause for concern, as they show the high level of parasitic contamination of the sandy beaches of the Rabat-Sale region. These areas constitute the main leisure options for adults and children, and point to the need for signage and information regarding appropriate public health risks, in order to avoid undesirable consequences.

Finally, *larva migrans* syndrome represents a global public health problem and remains neglected in many countries (**Silva *et al.*, 2000; Laggagio *et al.*, 2001; Thomé *et al.*, 2008**). The intervention of the public service in order to preserve animal health and the well-being of the population is a great challenge (**Reichmann *et al.*, 2000; Despommier, 2003; Cáceres *et al.*, 2005**). Given the high incidence of parasites zoonotic potential in the soil of the public areas of the beaches of the region of Rabat-Sale, it is necessary to emphasize the importance of adopting educational measures and dog and cat breeding control to reduce the risk of exposure to those parasites that cause zoonoses in adults and especially children. Health and environmental education must become a requirement for human users of beaches, including the presence of domestic animals in these places and the need to remove their faecal deposits and dispose of them properly.

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