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Longitudinal clinical and serological survey of abdominal angiostrongyliasis in Guaporé, southern Brazil, from 1995 to 1999

Estudo longitudinal clínico-sorológico da angiostrongilíase abdominal em Guaporé, sul do Brasil, de 1995 a 1999

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ABSTRACT

Abdominal angiostrongyliasis is a zoonotic infection caused by Angiostrongylus costaricensis, *a nematode with an intravascular location in the mesentery. Our objective was to address several aspects of the natural history of this parasitosis, in a longitudinal clinical and seroepidemiological study. A total of 179 individuals living in a rural area with active transmission in southern Brazil were followed for five years (1995-1999) resulting in yearly prevalence of 28.2%, 4.2%, 10%, 20.2% and 2.8% and incidences of 0%, 5.9%, 8% and 1.5%, respectively. Both men and woman were affected with higher frequencies at age 30-49 years. In 32 individuals serum samples were collected at all time points and IgG antibody reactivity detected by ELISA was variable and usually persisting not longer than one year. Some individual antibody patterns were suggestive of re-infection. There was no association with occurrence of abdominal pain or of other enteroparasites and there was no individual with a confirmed (histopathologic) diagnosis. Mollusks were found with infective third-stage larvae in some houses with an overall prevalence of 16% and a low parasitic burden. In conclusion, abdominal angiostrongyliasis in southern Brazil may be a frequent infection with low morbidity and a gradually decreasing serological reactivity.*

***Key-words*:** *Abdominal angiostrongyliasis.* Angiostrongylus costaricensis. *Abdominal pain. Eosinophilic gastroenteritis. Zoonosis.*

RESUMO

Angiostrongilíase abdominal é uma zoonose causada pelo Angiostrongylus costaricensis, *nematódeo que se localiza no interior de vasos mesentéricos. Nosso objetivo foi de abordar vários aspectos da história natural da parasitose, num estudo longitudinal clínico-sorológico. Um total de 179 indivíduos residentes em área rural no sul do Brasil, com transmissão ativa, foram seguidos por cinco anos. Neste período foram registradas prevalências de 28,2%, 4,2%, 10%, 20,2% e 2,8% e incidências de 0%, 5,9%, 8% e 1,5%. Tanto o sexo masculino quanto o feminino foram afetados com maiores frequências na faixa etária dos 30 aos 49 anos. Em 32 indivíduos, amostras de soro foram coletadas em todas as etapas e a reatividade de IgG detectada por ELISA foi variável e geralmente não persistindo mais do que um ano. Alguns padrões individuais foram sugestivos de re-infecção. Não houve associação com a ocorrência nem de dor abdominal nem com outras enteroparasitoses e não houve nenhum caso com diagnóstico confirmado (histopatológico) da infecção. Moluscos foram encontrados portando larvas infectantes de terceiro estadio, em algumas moradias, com uma prevalência geral de 16% e baixas cargas parasitárias. Em conclusão, a angiostrongilíase abdominal no sul do Brasil pode ser uma infecção frequente, porém com baixa morbidade e reatividade sorológica de gradual declínio.*

***Palavras-chave*s:** *Angiostrongilíase abdominal.* Angiostrongylus costaricensis. *Dor abdominal. Gastroenterite eosinofílica. Zoonose.*

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Angiostrongylus costaricensis is a metastrongylid nematode, parasitic of wild rodents. The worms live inside the mesenteric arterial system and terrestrial mollusks are intermediate hosts[13]. Human accidental infection may result in abdominal disease that has already been reported from most countries of Central and South America[14]. Diagnosis is only established through anatomicopathological examination of surgical or biopsy specimens, since there is no evidence of larval elimination in the feces, as seen with experimental infection of rodents[6 13]. Results from cross-sectional studies have been published[5], including an outbreak in Guatemala[9], besides many case reports from single or small groups of patients[2 14 16]. In 1995, a 41-year-old woman was diagnosed with abdominal angiostrongyliasis, living in a rural area of southern Brazil. That was the opportunity for attempting a first description of the natural history of this parasitosis, in a population-based longitudinal study.

MATERIAL AND METHODS

Colombo-Usina is a rural community in the municipality of Guaporé (52°08' W; 29°02'S) in the southernmost State of Rio Grande do Sul, Brazil. This area was selected after the histopathological diagnosis of abdominal angiostrongyliasis, with demonstration of an intra-arterial nematode, in a local resident. Adult worms of *Angiostrongylus costaricensis* were also recovered from mice experimentally infected with larvae found in slugs collected in the immediate surroundings of the house.

The houses in the community are distributed widely apart along a secondary road leading to the neighboring town of Anta Gorda. Local residents were registered and the houses were plotted on a map, in June 1995. At this time, informed consent to participate in the study was obtained from each individual. The investigation protocol was approved by the ethical committee (CEP-PUCRS, Process number 01/96) and performed according to Brazilian regulations (Decree MS-CNS 196/96). Collection of sera and fecal samples were performed in July 1996, March 1997, March 1998, October 1998 and March 1999.

Clinical evaluation (July 95, March 97 and March 98) was done by a questionnaire focused on the identification and description of abdominal pain. A brief physical examination was undertaken looking for vital signs and abdominal examination when abdominal pain was referred at that moment.

Serum samples were kept on ice until storage at -20°C in the laboratory. ELISA-IgG (76% sensitivity; 91% specificity) was performed as described elsewhere[4]. Briefly, the plates were sensitized with crude antigen of female worms at a concentration of 7μg/ml and serum samples were diluted 1:500 in 0.05% Tween 20, 5% skimmed milk PBS.

For the detection of *Strongyloides stercoralis* larvae in stools, a modified Baermann method was used[7]. Approximately 5g of fresh fecal samples were placed on four layers of surgical gauze supported by a plastic mesh, partially immersed in tap water in a plastic funnel and left overnight at room temperature. Larvae found under the stereomicroscope were identified through examination at a higher magnification under the microscope. For the detection of helminth eggs and protozoan cysts, part of the fecal samples were mixed with a 10% formalin solution and processed in the laboratory by Ritchie method[17].

For the detection of *Enterobius vermicularis* eggs, the method of Graham was performed. After detailed oral and written instructions, the individuals collected the samples by application of a transparent adhesive tape in the perineum early in the morning and placed the used tape over a glass slide. Slides were searched for eggs under the microscope.

For the study on intermediate hosts in the area, houses were randomly assigned as collection sites. Three collection sites were chosen in the lower and another three in the upper section of the main road. Terrestrial mollusks were collected during the same night by a screening of the surroundings of the houses by two researchers, beginning at 9 p.m. for 30 minutes. All mollusks found on the surface of the soil or vegetables were collected and kept in a cotton bag with grass until examination in the laboratory. The slugs were eviscerated and the body was minced and incubated in a 30% (w/v) pepsin (P-7152, Sigma) 0.07% (v/v) hydrochloric acid solution, overnight at room temperature. The preparations were then placed in a Baermann funnel for sedimentation and identification of metastrongylid larvae. A sample was inoculated *per os* in albino mice for the identification of adult worms inside the mesenteric arteries. The slugs were identified by external characteristics and if necessary by microscopic examination of genitalia, with the expert supervision of Dr. Georgina Mansur (Malacology Laboratory, PUCRS).

RESULTS

At least one set of data was collected for 179/198 individuals living in the area. These presented the following demographic characteristics: 83 males, 96 females, 33 ±18 years-old and age distribution shown in Figure 1. Prevalence of antibodies against *A. costaricensis* antigen ranged from 2.8% to 28 % and incidence from 0% to 8%, with the distribution shown in

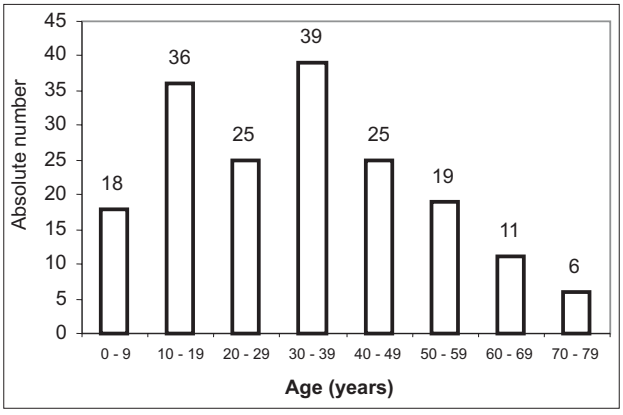

Figure 1 - Age distribution of the study population at Linha Colombo-Usina, Guaporé, southern Brazil.

Table 1 and Figure 2. The overall highest total number of seropositive individuals were detected from 30 to 49 years of age (Figure 3). When there was a substantial number of individuals with positive serology, male and female individuals were found to be equally affected: 1:0.95 and 0.83:1 in July 1995 and October 1998, respectively.

Table 1 - Abdominal angiostrongyliasis in Guaporé, RS, Brazil. Longitudinal serological study, from July 1995 to March 1999.

Date	Positive serology	Total Examined	Prevalence (%)	Incidence (%)
July 1995	39	138	28.2	-
March 1997	4	94	4.2	0
March 1998	9	90	10.0	5.9
October 1998	22	109	20.2	8.0
March 1999	2	69	2.8	1.5

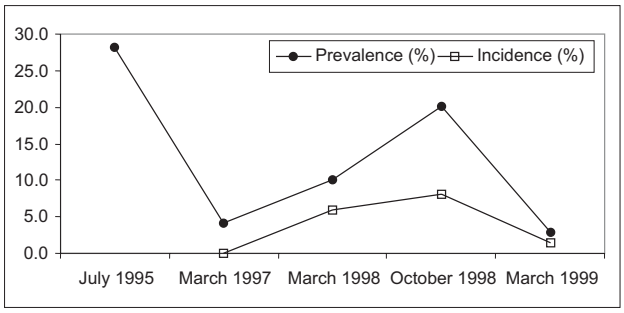

Figure 2 - Prevalence and incidence of abdominal angiostrongyliasis as indicated by serum antibodies anti-*Angiostrongylus costaricensis *in IgG-ELISA in Linha Colombo-Usina, Guaporé, southern Brazil.

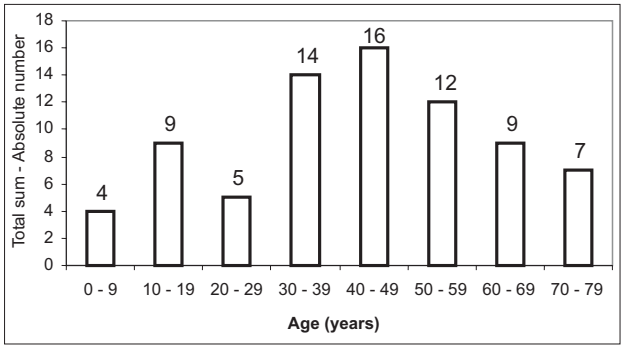

Figure 3 - Age distribution of serologically positive individuals among the population studied from 1995 to 1999, in Linha Colombo-Usina, Guaporé, southern Brazil.

Serological follow-up for 5 years was possible in 32 individuals (Table 2): 19/32 (40.6%) had persistently negative serology while only 1/32 individuals had persistently positive serology. This distribution is also seen within the group followed for 4 years (data not shown). Individual serological reactivity shows both variability and diversity of patterns (Figure 4). Presence of abdominal pain was reported by 38, 23 and 14 individuals in January 1996, March 1997 and March 1998, respectively, without a strong association with the serological reactivity seen in the ELISA (Table 3).

Ascaris lumbricoides and *Giardia intestinalis* were the most frequently found enteroparasites and situations of co-infection are shown in Tables 4 and 5. Occurrence of enteroparasites and serological reactivity to *A. costaricensis* in

Table 2 - Longitudinal study of abdominal angiostrongyliasis in Guaporé, RGS, Brazil, from July 1995 to march 1999. Serology results in 32 individuals examined at all 5 time points.

July 95	March 97	March 98	Oct 98	March 99	Totals
Neg	Neg	Neg	Neg	Neg	19
+	Neg	Neg	Neg	Neg	4
+	Neg	Neg	+	Neg	3
+	Neg	+	+	Neg	2
+	+	Neg	+	Neg	1
+	+	+	+	Neg	1
+	+	+	+	+	1
Neg	Neg	Neg	Neg	+	1
					Total = 32

Table 3 - Longitudinal survey on abdominal angiostrongyliasis in Linha Colombo-Usina, Guaporé, RS, southern Brazil, from 1995 to 1999. Estimation of association (Yule's coefficient) between abdominal pain (Abd pain) and positive serology.

Dates and occurrence	ELISA results			
of abdominal pain	Positive	Negative	Total	Y (Yule's association coefficient)
January 1996				
Abd pain +	9	29	38	
Abd pain -	19	51	70	
Total	28	80	108	
				- 0.09
March 1997				
Abd pain +	1	22	23	
Abd pain -	4	55	59	
Total	5	77	82	
				- 0.23
March 1998				
Abd pain +	3	11	14	
Abd pain -	6	66	72	
Total	9	77	86	
				+ 0.5

Table 4 - Longitudinal survey on abdominal angiostrongyliasis in Linha Colombo-Usina, Guaporé, RS, southern Brazil, from 1995 to 1999. Coproparasitological survey (Ritchie method) performed in 1995.

Diagnosis	Isolated infections	%	Multiple infection	%	Total	%
Giardia intestinalis	4	5.0	1	1.3	5	6.3
Ascaris lumbricoides	1	1.3	4	5.0	5	6.3
Iodamoeba sp	3	3.8	0	0.0	3	3.8
Trichuris trichiura	0	0.0	2	2.5	2	2.5
Entamoeba coli	1	1.3	1	1.3	2	2.5
Strongyloides stercoralis	0	0.0	1	1.3	1	1.3
Taenia sp	0	0.0	1	1.3	1	1.3
Enterobius vermicularis	1	1.3	0	0.0	1	1.3
Entamoeba hartmani	1	1.3	0	0.0	1	1.3
Negative	64	80.0	0	0.0	64	80.0
Total	**75**	**93.8**	**10**	**12.5**	**85**	**106.3**
Multiple infections						
A. lumbricoides + T. trichiura	2					
A. lumbricoides + S. strongyloides	1					
A. lumbricoides + Taenia sp	1					
G. intestinalis + E. coli	1					
Total	5					
			Corrected total		**80**	**100.0**

1995 and 1997 were not significantly associated (Yule's association coefficient = -0.11 and 0.12, respectively for 1995 and 1997 data). Prevalence of strongyloidiasis, as evaluated by the Baermann method, was 10% and 4% with an incidence of 4.2% in March 1997. In July 1996, a search for enterobiasis showed a prevalence of 4.8% (3/63).

The nocturnal search for terrestrial mollusks was successful in 4/6 sites (Table 6) and metastrongylid larvae were found in 10/60 mollusks, mainly slugs (*Phyllocaulis variegatus*) from the Veronicelid family. The spatial distribution of the collected mollusks within each focus is shown in Figure 5. In house number 30, where no slug was found in the nocturnal search, a dead slug was found by the peasant inside the well from where water is pumped for human consumption.

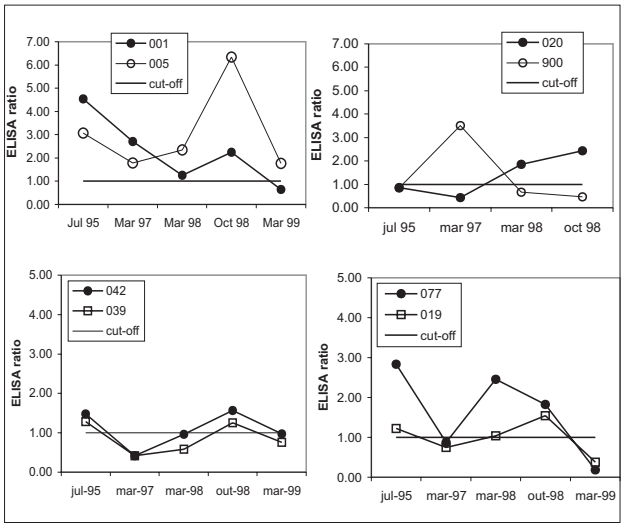

Figure 4 - Individual serological reactivity (IgG anti-*Angiostrongylus costaricensis*) along several time points, from 1995 to 1999, in Linha Colombo-Usina, Guaporé, southern Brazil. The individual 005 shows a striking increase in humoral reactivity, suggestive of re-infection. Individuals 001, 042, 039, 077 and 019 also presented a pattern suggestive of re-infection with a variable but declining reactivity. Both 020 and 900 show seroconversion with persistence of reactivity up to October 1998.

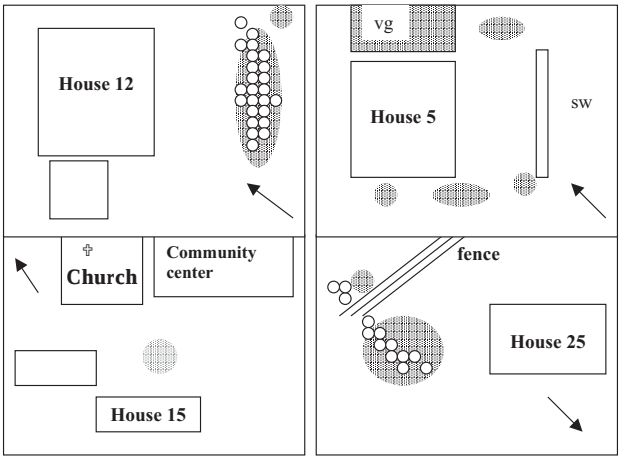

Figure 5 - Schematic drawing of the surroundings of 4 out of the 6 houses where a nocturnal search for terrestrial mollusks was undertaken in Linha Colombo-Usina, Guaporé. Mollusks were found in shadowed areas, illustrating the focal distribution of potential A. costaricensis vectors. Small circles in shadowed areas (houses 12 and 25) represent piles of stones. At house 5, there was a stone wall (sw) and "vg" is the vegetable garden. At house 15 in the center of the shaded area there is a small bushy tree. Arrows point North.

Table 5 - Longitudinal survey on abdominal angiostrongyliasis in Linha Colombo-Usina, Guaporé, RS, southern Brazil, from 1995 to 1999. Coproparasitological survey performed (Ritchie method) in 1997.

Diagnosis	Isolated infections	%	Multiple infection	%	Total	%
Ascaris lumbricoides	10	11.7	8	9.4	18	21.7
Entamoeba coli	6	7.0	2	2.3	8	9.4
Trichuris trichiura	3	0.0	6	7.0	9	10.5
Enterobius vermicularis	2	2.3	0	0.0	2	2.3
Taenia saginata	1	1.1	0	0.0	1	1.1
Ancylostomatic larvae	0	0.0	1	1.1	1	1.1
Giardia intestinalis	0	0.0	1	1.1	1	1.1
Strongyloides stercoralis	0	0.0	1	1.1	1	1.1
Hymenolepis nana	0	0.0	1	1.1	1	1.1
Negative	63	74.1	0	0.0	63	74.1
Total	**85**	**100.0**	**20**	**23.5**	**105**	**123.5**
Multiple Infections						
A. lumbricoides + *T. trichiura*	5					
A. lumbricoides + *H. nana*	1					
A. lumbricoides + ancylostomatic larvae	1					
A. lumbricoides + *S. stercoralis*	1					
G. intestinalis + *E. coli*	1					
T. trichiura + *E. coli*	1					
Total	**10**					
		Corrected Total			**85**	**100.0**

Table 6 - Presence of metastrongylid larvae in terrestrial mollusks collected in immediate surroundings of 6 houses in Linha Colombo Usina, Guaporé, RS, Brazil, in March 1999.

Mollusk species	Site	nº	Infected (%)	Prevalence	Parasitic burden
Phyllocaulis variegatus					
	15	24	4	16.7	3, 2, 1, 2
	25	12	2	16.7	3, 103
	12	8	0	0.0	
	5	10	3	30.0	2, 10, 13
Bradybaena similaris	5	5	1	20.0	36
Limax flavus	5	1	0	0.0	
Total		**60**	**10**	**16.7**	

DISCUSSION

Longitudinal population-based studies are important for a better evaluation of the epidemiology and natural history of infection and disease. Most of the knowledge about human infection with *Angiostrongylus costaricensis* comes from individual or small series reports of surgically treated individuals[14]. In southern Brazil, surgical intervention due to intestinal perforation or obstruction related to abdominal angiostrongyliasis is more frequent than other complicated inflammatory bowel diseases, such as ulcerative colitis and tuberculosis[1]. But the more severe clinical courses in infectious diseases in general are usually only *the tip of the iceberg*.

Older editions of some textbooks[3] present abdominal angiostrongyliasis as a pediatric problem, based on a report of a case series from a pediatric hospital, that was published twice updating a growing but cumulative number of children[11 12].

In southern Brazil, it has been previously demonstrated that the parasitosis affects adults as well as children[6]. The present results show an overall predominance of adults with positive serology for abdominal angiostrongyliasis.

Variability in prevalence as seen in the present report may be interpreted as resulting from focality and seasonality, two main characteristics of zoonotic infections. A higher prevalence in 1995 is coincident with the occurrence of one complicated clinical course that was taken as the index case for the study. This fact may also result from a higher probability for severe cases to occur among an increased number of infected people at the time.

Individual follow-up of antibody reactivity as demonstrated by IgG-ELISA has shown indications for two main possibilities. First, abdominal angiostrongyliasis appears not to be a persistent infection. Most of the documented peaks in serological reactivity did not last more than a year and only a few individuals had persistent positive serology. These data further support the presumption that man is not a well-adapted host for *A. costaricensis*, based on the pathological reports and on the failure to detect L1 larvae in feces. Secondly, most infections are asymptomatic. Not a single individual presented a very suggestive clinical picture of severe abdominal disease that would include at least an acute course of abdominal pain and fever. Most episodes of abdominal pain were transient or probably associated with viral or bacterial gastroenteritis.

Although not yet properly investigated, serological cross-reactions with antigens from *A. costaricensis* and other parasites are known to occur[4]. Other intestinal parasitoses were detected in the population but no significant association with the anti-*A. costaricensis* antibody reactivity was detected. This is not against the idea that there are cross-reactive epitopes, since intensity of the nonspecific response may be low depending on the chronicity or parasitic burden of the heterologous infections[10]. Co-infections or multiple infections should be better investigated in order to clarify all the missing bricks in the knowledge of humoral cross-reactivity among diverse combinatory situations.

The relatively low prevalence of other intestinal parasitosis is not a surprise, since local peasants, mostly descendents of Italian immigrants, have good sanitary conditions and educational level. The finding of a dead slug in the source of water for human consumption in one of the houses, adds support to the hypothesis of water transmission of *A. costaricensis*. This possible route for transmission was previously commented by Ubelaker et al[18] reporting the emergence of third-stage larvae from experimentally infected aquatic snails.

Several mollusk species are susceptible to *A. costaricensis* infection but only a few have a significant role as hosts, such as the veronicelid slugs[15]. Infection was detected mainly in *Phyllocaulis variegatus*, the first intermediate host described in Brazil[8] with prevalence and parasitic burdens in the range as previously reported[15]. Mollusks were usually found restricted to one area next to the houses, where piles of stones, bricks or hay, and soil cracks had provided shelter, as seen in Figure 5. This focality in the distribution of the intermediate host may be important to determine the absence of widespread infection in the human population.

In conclusion, abdominal angiostrongyliasis in southern Brazil appears to occur with low, fluctuating prevalence and incidence, and low morbidity. Although it is not a major public health problem, infection may be regularly detected, with a predominantly asymptomatic course, usually leading to spontaneous remission.

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