

## Prevalence of intestinal nematodes in alcoholic patients

Frequência de nematóides intestinais em pacientes alcoólatras

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**Abstract** We report the results of a retrospective study on the frequency of intestinal nematodes among 198 alcoholic and 440 nonalcoholic patients at the University Hospital Cassiano Antonio Moraes in Vitória, ES, Brazil. The control sample included 194 nonalcoholic patients matched according to age, sex and neighborhood and a random sample of 296 adults admitted at the same hospital. Stool examination by sedimentation method (three samples) was performed in all patients. There was a significantly higher frequency of intestinal nematodes in alcoholics than in controls (35.3% and 19.2%, respectively), due to a higher frequency of *Strongyloides stercoralis* (21.7% and 4.1%, respectively). Disregarding this parasite, the frequency of the other nematodes was similar in both groups. The higher frequency of *S. stercoralis* infection in alcoholics could be explained by immune modulation and/or by some alteration in corticosteroid metabolism induced by chronic ethanol ingestion. Corticosteroid metabolites would mimic the worm ecdysteroids, that would in turn increase the fecundity of females in duodenum and survival of larvae. Consequently, the higher frequency of *Strongyloides* larvae in stool of alcoholics does not necessarily reflect an increased frequency of infection rate, but only an increased chance to present a positive stool examination using sedimentation methods.

**Key-words:** Alcoholism. Strongyloidiasis. *Strongyloides stercoralis*. Intestinal nematodes.

**Resumo** Foi feito um estudo retrospectivo da frequência de nematóides intestinais em 198 alcoolistas e em 440 controles, não alcoólatras, atendidos no Hospital Universitário C.A. Moraes, em Vitória, ES. O grupo controle foi formado por 194 pacientes não alcoólatras, pareados por idade, sexo e procedência, e por 296 pacientes adultos, internados no mesmo hospital, escolhidos aleatoriamente. Fez-se exame parasitológico pelo método de sedimentação em todos os casos. Houve uma frequência significativamente maior de nematóides intestinais no grupo de alcoólatras do que nos controles (35,3% e 18,7%, respectivamente), devido a frequência maior de *Strongyloides stercoralis* (21,7% e 4,1%, respectivamente). A frequência dos outros nematóides foi semelhante nos dois grupos. A maior frequência de *S. stercoralis* nos alcoólatras poderia ser explicada pela imunomodulação induzida pela ingestão abusiva de etanol e/ou por alteração do metabolismo dos corticosteróides induzidas pelo etanol, aumentando a quantidade de metabólitos que podem mimetizar os ecdisteróides do verme, aumentando a fecundidade das fêmeas no duodeno e a sobrevivência das larvas. Desse modo, a maior frequência de *S. stercoralis* nos alcoólatras não refletiria um real aumento na prevalência, mas sim um aumento na positividade do exame parasitológico devido ao aumento do número de larvas rabdtóides eliminadas pelas fêmeas no duodeno.

**Palavras-chaves:** Alcoolismo. *Estrongiloidíase*. *Strongyloides stercoralis*. Nematóides intestinais.

In developing countries, both chronic alcoholism and intestinal worms are common, but the frequency and severity of worm infection in alcoholics have not been studied. Experimental observations showed that rats treated with ethanol and infected with *Trichinella spiralis* showed a significant delay in expulsion of intestinal

worms compared with the control group<sup>16</sup>. Also in rats treated with ethanol and infected with *T. spiralis*, there was a reduction in the number of blood neutrophils and eosinophils and a decreased production of IFN $\gamma$  by mesenteric lymph node cells at the early phase of infection, when compared with infected rats not treated

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with ethanol<sup>12</sup>. According to the authors, the reduction of inflammatory cells and cytokines (IFN $\gamma$ ) would be the main factors related to the increased survival and longevity of adult worms in rats treated with ethanol<sup>11</sup>. On the contrary to these observations in rats, mice chronically consuming ethanol that were infected with larvae of *Strongyloides stercoralis* presented migration of eosinophils to parasite's microenvironment and produced antibodies at levels equivalent to those seen in control mice<sup>7</sup>.

There is one observation reporting high frequency of *Strongyloides stercoralis* in patients with alcoholic cirrhosis<sup>4</sup>. However, the authors refer to high frequency of the helminth in cirrhosis of any etiology, without discussion about the possible mechanisms involved in

this association. They admitted that liver cirrhosis, but not alcoholism, could be the main factor responsible for the increased frequency of *S. stercoralis* in the samples studied.

Alcoholism and helminth infection are both frequent among people seeking medical care at University Hospital Cassiano A. Moraes, in Vitória, State of Espírito Santo, Brazil. As ethanol abuse induces behavior changes that enhance contact with infectious agents<sup>1</sup>, we hypothesize that intestinal nematodes would be more prevalent among alcoholic patients. To test this hypothesis we decided to investigate the frequency of intestinal nematodes among alcoholics attending an outpatient unit (Alcoholism Unit, Gastroenterology Division) and among non-alcoholic individuals attending the same Hospital.

## PATIENTS AND METHODS

In a retrospective study, the records of 198 patients from the Alcoholism Unit of the University Hospital C.A. Moraes were reviewed and the results of stool examinations were retrieved. All patients with daily ingestion higher than 80mg for men and 60mg for women were considered to be alcoholics. For comparison, the results of stool examinations were retrieved from the records of two groups of non-alcoholic patients attended at the same Hospital during the same period. One control group of 144 adult in-patients with similar age and gender distribution, and living in the same neighborhoods, was considered as partially matched control group. The other control group was a random sample of 296 fecal examinations of adult inpatients, resident in different neighborhoods of Metropolitan Vitoria, considered as a control for the frequency of

intestinal nematodes in adult patients that attend at the University Hospital. In the alcoholic group and in the two control groups, fecal examination was done by sedimentation method in three samples for each patient and at the same laboratory.

The frequency of *Strongyloides stercoralis* and other nematodes (*Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Ancylostoma duodenale* and *Necator americanus*) were registered and compared.

Epilinfo version 2000 software was used to calculate the Odds Ratio and the Chi-square or Exact Fisher Test to verify the strength of associations or differences between frequencies. Values of p less than 0.05 were considered statistically significant.

The ethical committee of the Federal University of Espírito Santo approved this research.

## RESULTS

In the alcoholic group, liver cirrhosis was present in 58 (29.3%) patients. Table 1 displays age and gender distribution together with frequency of intestinal nematodes in alcoholic and in two control groups. The frequency of at least one intestinal nematode is significantly higher among alcoholics than in the two control groups. When considered separately the frequency of *S. stercoralis* is significantly higher in alcoholics than in controls. However there was no significant difference between alcoholics and controls when we compared the frequency of the other intestinal

nematodes grouped together as other nematodes (Table 1). The frequencies of other nematodes for alcoholics and the matched control group were, respectively: *A. lumbricoides* 6.8 and 7.3%, *T. trichiuris* 3.1 and 4.6% and *Ancilostomidae* 2.2 and 2.5%. Thus, the increased frequency of intestinal worms in alcoholics was due to an increased frequency of *S. stercoralis*, but not of other worms. In addition, there was no significant difference in the frequency of worms between alcoholics with or without cirrhosis (respectively: 37.9% and 34.2% for at least one nematode and 25.8% and 20% for *S. stercoralis*).

## DISCUSSION

The results presented here demonstrated that there is a significant higher frequency of intestinal nematodes in alcoholics, with or without cirrhosis, than in control groups and that this difference was due to the higher frequency of *S. stercoralis*.

Although we did not investigate all the individual socioeconomic parameters, the two groups, cases and controls, belong to the same socioeconomic conditions,

since the University Hospital is a public hospital that offers medical care to people from low socioeconomic class. In addition, the patients of one of the control groups came from the same neighborhoods as the alcoholics and did not differ in age or gender. Although cases and one control group showed no differences in relation to age and gender, we could not rule out several cofounders that are frequent when using in-patient

Table 1- Age, gender and frequency of intestinal nematodes in alcoholics and in two control samples of non alcoholics at the University Hospital Cassiano A. Moraes, Vitória, ES, Brazil.

Variables	Groups		OR (95% CI)	P
	alcoholic (n=198)	control ( n <sup>a</sup> =144)/( n <sup>b</sup> =296)		
Age (mean ± sd)	44.6 ± 10.3	39.3 ± 15.3 <sup>a</sup>	nd	0.131
Gender (M/F)	180/18	124/20 <sup>a</sup>	nd	0.222
At least one nematode n (%)	70 (35.3)	27 (18.7) <sup>a</sup>	2.37 (1.38-4.08)	0.001
<i>Strongyloides stercoralis</i> n (%)	43 (21.7)	57 (19.2) <sup>b</sup>	2.32 (1.51-3.57)	0.000
		7 (4.9) <sup>a</sup>	5.43 (2.25-13.70)	0.000
		21 (7.1) <sup>b</sup>	3.63 (2.01-6.59)	0.000
Other nematodes n (%)	27 (13.6)	20 (13.9) <sup>a</sup>	0.98 (0.50-1.91)	0.940
		39 (13.1) <sup>b</sup>	1.04 (0.59-1.82)	0.499

<sup>a</sup> Control sample matched by age, gender and neighborhood. <sup>b</sup> Random sample of unmatched controls representing the prevalence of intestinal nematodes in non alcoholic adult patients at the University Hospital.

samples. Thus, all the conclusions may consider the caveat resulting the assumption that the two samples, cases and controls, are comparable.

We also know that the Hoffman's method used for fecal examinations possibly leads to underestimation of *Strongyloides* infection and apparently could be a confounding factor. However, it is important to remind that this occurred for both the cases and the controls, what adjusted this factor to the two groups.

There was no significant difference in frequency of intestinal nematodes between alcoholics with or without cirrhosis. This observation indicates that ethanol ingestion is in relationship with the increased risk for *Strongyloides* infection, but not only liver cirrhosis, as admitted by Gaburri et al<sup>4</sup>.

We have no explanation for the increased frequency of *S. stercoralis* infection in alcoholics. If the behavioral changes induced by ethanol abuse constitute a risk for helminth infection, it would be expected an increased risk for all nematode infection. However, in this observation only *S. stercoralis* infection in alcoholics was significantly higher than in controls. Factors other than behavior changes linked to chronic ethanol ingestion may be responsible for this increased frequency of *Strongyloides* in fecal examinations of alcoholics.

Immune response, both innate and adaptive, may be impaired in alcoholics and both mechanisms play an important role on resistance against nematodes<sup>16,9,17,18</sup>. The resistance against helminthes, mainly demonstrated by the delay in the expulsion of adult worms in experimental models in rodents, is dependent on IL-4 and IL-13, typical Th2 cytokines<sup>3,13</sup>. Alcohol consumption decreases the production of Th1 cytokines, but the production of Th2 cytokines is normal or increased<sup>17,18</sup>. Therefore, alcohol intake induces an immune modulation with a shift toward Th2 cytokines, thus it is difficult to blame this effect of ethanol on the decreased resistance to *S. stercoralis*, since this resistance is, at least in part, dependent on Th2 responses. However, the impairment of Th1 responses and reduction in number and function of inflammatory cells would interfere with some steps in the mechanisms of resistance against nematodes in

which inflammatory process is a relevant factor<sup>10</sup>. It has been demonstrated that TNF- $\alpha$  is important in interleukin 13-mediated protective Th2 responses during helminth infection<sup>2</sup>.

The survival of *S. stercoralis* in the gut may be influenced by other factors than immune response. Genta<sup>5</sup> raised the hypothesis that glucocorticoids and corticoids metabolites would enhance the fecundity of *S. stercoralis* females in duodenum and the maturation of rhabditiform larvae in the gut. He based his hypothesis on the fact that disseminated strongyloidiasis is more frequently associated with use of corticoids than with other kinds of immunosuppression. The ecdysteroids, hormones that regulate ecdyses in larvae of helminthes and other invertebrates<sup>11</sup>, have structural similarities with corticosteroids and other steroid metabolites detected in human serum of normal, nonparasitized individuals<sup>8</sup>. Thus, an increased level of corticoids or its metabolites would mimic the ecdysteroids, increasing the fecundity in females and the maturation of larvae from rhabditiform to filariform stage, thereby enhancing autoinfection.

We propose that Genta's hypothesis could explain the high frequency of *S. stercoralis* larvae in fecal examination of alcoholics, because ethanol ingestion interferes with the metabolism of steroids in two ways: a) interfering with the hypothalamic-pituitary-adrenal axis; and b) interfering with steroids metabolism in the smooth endoplasmic reticulum in the liver. Acute ingestion of ethanol increases the plasma levels of ACTH and corticosterone, it also occurs, in lower levels, in chronic ethanol abusers<sup>14</sup>. Chronic alcohol ingestion induces the mixed-function oxidase system, consequently interfering with the metabolism of steroids. These alterations in steroid metabolism occur even before liver cirrhosis, but are more evident in alcoholics with cirrhosis.

Taking into account the hypothesis proposed by Genta and the possible alterations in corticosteroids metabolism in alcoholics, one may admit that the high frequency of *Strongyloides* larvae in the stool of alcoholics could be due to an excessive amount of corticosteroids or corticoid metabolites in the blood. The

excess of hormones or its metabolites would increase the fecundity of females in duodenum, thus it would result in an increased number of rhabditiform larvae in the gut and the probability that they may be found during fecal examination. In this manner, the higher frequency of *Strongyloides* larvae in stool of alcoholics would not reflect an increased frequency of infection, but only an

increased chance to present positive stool examination using sedimentation methods.

A matched case-control study, using the more sensitive Baermann's method to detect and quantify *Strongyloides* larvae, is in progress in our laboratory to clarify the relationship between abusive ethanol ingestion and *Strongyloides stercoralis* infection.

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