HIGH PREVALENCE OF *STRONGYLOIDES STERCORALIS* AMONG FARM WORKERS ON THE MEDITERRANEAN COAST OF SPAIN: ANALYSIS OF THE PREDICTIVE FACTORS OF INFECTION IN DEVELOPED COUNTRIES


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Abstract. Strongyloidiasis is a parasitosis characterized by persistent infection before dissemination and the development of potentially fatal disease. Since diagnosis is difficult, knowledge of the prevalence and geographic distribution of the disease is of practical importance. A study was made of *Strongyloides stercoralis* infection in a random and representative sample of farm workers in a tourist region in Spain based on the detection of larvae of triple stool samples. The prevalence of infection was 12.4% (95% confidence interval [CI] = 8.4–16.4). None of the 26 clinical or epidemiologic variables analyzed were found to be predictive of infection. Only eosinophilia (> 400 eosinophils/mm$^3$) was significantly greater among the infected individuals (odds ratio = 73.4, 95% CI = 16.3–327.0), with a sensitivity of 93.5% and a specificity of 93.1%. A screening program is proposed to detect eosinophilia, to provide treatment without stool examinations, and thus afford a cost-effective policy for preventing the development of severe forms of the disease among specific risk groups where the prevalence of other parasitoses is low.

INTRODUCTION

*Strongyloides stercoralis* is the only nematode with an endogenous cycle that infects humans for many years without requiring further exposure to contaminated soil, a situation characterized by few or no symptoms, and which therefore may go unnoticed. Occasionally, the autoinfection cycle can accelerate and give rise to a so-called hyperinfection syndrome that can prove fatal if adequate therapy is not provided.\(^1\)

Infection with *S. stercoralis* is one of the most difficult parasitic infections to diagnose,\(^2,3\) and effective treatment consists of total parasite eradication before fatal complications develop.\(^4\) In this context, strongly suspected infection in any patient who had visited endemic regions is a key aspect in successful diagnosis, management, and treatment. Knowledge of the areas where the disease is endemic is therefore of considerable practical importance.\(^5\)

Isolated cases of disseminated strongyloidiasis have been reported in Spain, although no endemic zones have been identified in this country.\(^6,7\) Since the Hospital of Gandia (in Valencia on the Mediterranean coast of Spain) opened in 1984, relatively frequent cases of strongyloidiasis have been documented among patients hospitalized for other reasons.\(^8,9\) A prospective clinical study was made over an eight-year period involving 152 infected patients (almost all farm workers). In this study, 13% presented with severe forms of the disease, and the mortality rate was 30%.\(^10\)

The study reported in this paper had three purposes: 1) to determine the prevalence of *S. stercoralis* among the farm workers (where the highest risk of infection has been recorded) in the area of Gandia, 2) to identify predictive factors of infection to implement appropriate disease prevention programs, and 3) to evaluate the efficacy and side effects of treatment of this disease with thiabendazole.

MATERIALS AND METHODS

The area of Gandia, part of the triple-province Valencian Community on the Mediterranean coast of Spain, has a population of 135,000 inhabitants. The mean per capita income is slightly greater than elsewhere in the European Union. Eighteen percent of the active population are farm workers (someone who consists of farm work), who characteristically live with their families under adequate hygiene-sanitary conditions and are not grouped into collective farms. The study subjects were selected from a random numbers table stratified into population centers based on the agricultural census of the area (13,756 individuals).

Due to the lack of previous studies in this region, a pilot survey of 90 subjects was conducted to obtain an approximate prevalence for calculating the corresponding sample size. *Strongyloides stercoralis* was detected in 10 individuals, yielding a prevalence of 11.1%. Accordingly, the sample size required to afford a precision of ± 4% was found to be 277 subjects. Taking into account that addresses in the population census may have varied, contact letters were mailed to 320 agricultural workers, explaining the reasons for the study in simple language, and requesting consent to participation. A stamped envelope was enclosed, containing a form on which the subject was requested to indicate a contact telephone number for arranging a visit. A member of our group then visited the subject to deliver a questionnaire containing the study variables, explain the stool collection method (stools deposited in the morning on three different days and submitted to the laboratory of the area hospital within two hours), and collect a blood sample for determining an eosinophil count in peripheral blood. The stools samples were immediately processed upon receipt by the microbiology laboratory: microscopic examination, formalin-ether concentration, and agar plate culture.

The diagnosis was based on detection of the rhabditiform larvae by the agar plate culture technique,\(^11\) which was performed by the same examiner and evaluated over three consecutive days. The microbiologist was blinded to the identity of the individual providing the stool specimens, and thus also to the results of the questionnaire. The eosinophil count was determined using a Hitachi 717 Coulter Counter (Boehringer Manheim, Mannheim, Germany). Eosinophilia was defined by a count > 500 cells/mm$^3$.
The following variables were studied: subject age and sex, municipality of residence, occupational status (active, inactive or retired farm worker), the number of years working on the land, the number of years elapsed since abandoning agricultural activities (in the case of retired subjects), type of crop (rice, citric fruits, vegetable plots, other crops), locality of agricultural activities, skin protection measures adopted while at work (barefoot or with scant foot protection, wearing of gloves), the consumption of non-potable irrigation water, hygienic conditions in the home (water suitable for human consumption, toilet draining into the public sewage system), habits of hygiene (toilet use, defecation in the fields, washing of hands after defecation), alcohol consumption (> 40 grams/day), tobacco use (> 20 packs of cigarettes/year), previous and present surgical interventions and diseases according to the International Classification of Diseases, Ninth Edition (ICD-9), past and present parasitoses, habitual medication (laxatives, antacids, Type 2 antihistamine receptors and other antihistamines, proton-pump inhibitors, astringents), past and present immunosuppressive drugs (corticosteroids, cytostatic agents, immunosuppressors), and symptoms not attributable to other concomitant disorders: respiratory (cough, wheezing, hemoptysis), cutaneous (larva currens, urticaria, generalized pruritis), and digestive (chronic or intermittent diarrhea, melena, abdominal pain, dyspepsia, heartburn, epigastric pain, constipation, nausea, vomiting, pruritis ani).

Infected individuals were treated with thiabendazole (25 mg/kg/day in two daily doses for five consecutive days). Four stool samples were requested one and three months after the end of treatment. Clinical data were related to drug intolerance and the improvement or disappearance of possible symptoms present before treatment were collected, and the eosinophil count was monitored.

This study was reviewed and approved by the Institutional Human Subjects Review Board of the Gandia Hospital.

Statistical analysis. The prevalence of *S. stercoralis* infection was determined as the proportion of individuals with positive stool samples. Corresponding 95% confidence intervals (CIs) were also calculated. The chi-square test was used for qualitative variables with application of Fisher’s exact test as required and the Student’s t-test for comparison of the means. The association between possible risk factors and infection was estimated in terms of the corresponding odds ratio (OR), with calculation of the 95% CI. Multivariate analysis was performed using logistic regression analysis. Calculations were made of the sensitivity, specificity, positive predictive value, and negative predictive value of the variables found to be significant. A receiving operator characteristic (ROC) curve was in turn used to establish the best cut-off points for these variables.

RESULTS

Seventeen contact letters were returned because the addresses were incorrect, and 28 subjects failed to reply. A total of 275 individuals agreed to participate in the study, though only 269 presented to the visit with the interviewer. Nineteen subjects sent no stool samples. Thus, a total of 70 (21.7%) individuals were lost to the study, and the final sample size comprised 250 subjects, although 12 refused blood collection for the eosinophil assay.

*Strongyloides stercoralis* was detected in 31 individuals, yielding a prevalence of 12.4% (95% CI = 8.4–16.4). No other parasite was identified with the techniques used. The demographic and epidemiologic characteristics of the study sample are shown in Table 1. Home hygiene was good in all cases. Eight individuals admitted to defecation in the fields. Similarly, no differences were recorded in terms of toxic habits, diseases, or medication considered related to *S. stercoralis* infection (Table 2). With regards to clinical manifestations, most subjects presented digestive symptoms compatible with *S. stercoralis* infection, with no predominance among the infected individuals (Tables 3).

In relation to the laboratory findings, eosinophilia was detected in 12.6% (95% CI = 9.6–15.6) of the 238 patients in which this parameter was measured (Table 3). Only seven uninfected individuals had eosinophil counts > 500 cells/mm$^3$ (range = 612–720 cells/mm$^3$), while most of the infected subjects showed eosinophilia (range = 620–1,560 cells/mm$^3$). Eosinophilia was the variable showing the greatest statistically significant difference ($P < 0.0001$) with respect to the *S. stercoralis*-infected group (OR = 133, 95% CI = 34–555, $P < 0.05$), with a sensitivity of 82%, a specificity of 96%, a positive predictive value of 78%, and a negative predictive value of 96%. The ROC curve showed a cut-off value of 400 eosinophils/mm$^3$ to yield the best sensitivity and specificity performance (93.5% and 93.1%, respectively, positive predictive value = 65%, negative predictive value = 98%). Multivariate analysis showed that only eosinophilia was associated with the presence of infection (OR = 73, 95% CI = 16.3–327.0).

The 31 infected individuals received treatment for their

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic and epidemiologic characteristics of <em>Strongyloides stercoralis</em> infection among agricultural workers in the area of Gandía, Valencia, Spain*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. stercoralis</em>-positive agricultural workers (n = 31)</td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>68.6 ± 8.0</td>
</tr>
<tr>
<td>Men</td>
<td>100%</td>
</tr>
<tr>
<td>Presently active worker</td>
<td>77%</td>
</tr>
<tr>
<td>Years working in fields</td>
<td>–</td>
</tr>
<tr>
<td>&lt; 20</td>
<td>10%</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>90%</td>
</tr>
<tr>
<td>Possible contagion route</td>
<td>–</td>
</tr>
<tr>
<td>Works barefoot/no protection</td>
<td>97%</td>
</tr>
<tr>
<td>Consumes non-potable water</td>
<td>94%</td>
</tr>
</tbody>
</table>

*NS = no significant difference found between groups.
infection. Only one subject (3.1%) failed to complete therapy due to intolerance (nausea and acute confusion manifestations). A post-treatment stool control sample showed no parasites in the remaining cases. Eosinophilia persisted in four individuals despite stool sample negativity, although in two of them the cell count decreased considerably. Sixteen of the 24 subjects with symptoms compatible with S. stercoralis infection were found to be asymptomatic after treatment.

DISCUSSION

The present study shows the prevalence of S. stercoralis infection in the area of Gandia (Valencia, Spain) to be 12.4% (95% CI = 8.4–16.4), with an increased frequency of the disease among elderly males. None of the risk factors studied proved to be significant for infection, and no data were obtained to suggest the existence of infection other than eosinophilia, with a sensitivity of 93.5% and a specificity of 93.1%. Treatment with thiabendazole (25 mg/kg/day for five days) was both effective and well tolerated.

The importance of identifying the existence of an endemic status in a specific area has been well established. Considering the characteristics of this infection and the consequent difficulty of conducting prospective surveys, reliable information on the true prevalence of S. stercoralis in this region is limited. Most studies conducted to date are heterogeneous, incomplete, and of a retrospective nature, reporting wide-ranging prevalences from 0.6% to 40%, with the disease being regarded as endemic in all of them.12–19

In 1992, the first study of the prevalence of intestinal parasites based on a representative sample of immigrant migrant workers on farms in North Carolina was published. Larvae of S. stercoralis were detected in 4% based on the microscopic examination of stools.20 Prospective studies in Africa, Brazil, and Southeast Asia have indicated prevalences ranging from 2% to 25% in diverse populations. However, similar studies have not been conducted in Europe.

The prevalence observed in our study is among the highest of all those described in this report. This is a remarkable finding given the fact that the study area was in a developed country where the conditions of hygiene are very different from those found in the previously mentioned studies. This observation could be explained by the fact that we used the more sensitive agar plate technique2,27,28 and analyzed three

| Clinical and laboratory findings of Strongyloides stercoralis infection among agricultural workers in the area of Gandia, Valencia, Spain* |
|-------------------------------------------------|-------------------------------------------------|-----------------|
| S. stercoralis-positive agricultural workers (n = 31) | S. stercoralis-negative agricultural workers (n = 219) | P |
| Eosinophils (mean ± SD) | 925.8 ± 378.5 (n = 31) | 210.6 ± 131.0 (n = 207) | < 0.00001† |
| Eosinophils (median) | 1,000 | 200 |
| No. of subjects with > 500 cells/mm³ | 83.7% | 3.3% | < 0.05, OR = 133, 95% CI = 34–555 |
| Global symptoms compatible with S. stercoralis | 77.4% | 71.6% | NS |
| Digestive symptoms compatible with S. stercoralis | 70.9% | 71.6% | NS |
| Skin symptoms compatible with S. stercoralis | 22.5% | 19.1% | NS |
| Pruritis ani | 16.1% | 22.8% | NS |

* OR = odd ratios; CI = confidence interval; NS = non-significant intergroup differences.
† The S. stercoralis-positive individuals exhibited a significantly higher peripheral eosinophil count.
stool samples per individual. The results obtained therefore suggest that the Valencian Mediterranean coastal region of Spain, which has a very important tourist industry, is effectively an endemic zone for *S. stercoralis*.

A number of studies have described different clinical and epidemiologic aspects associated with *S. stercoralis* infection, although few have addressed the specific influence of each of these factors in relation to infection or as predictors of the existence of chronic infection. In our study, as reported by Loutfy and others and Buchwald and others, we detected no statistically significant risk factor, with the exception of a slight increase in risk associated with advanced age and male sex. The small size of the infected group may have influenced our results, although the series was nevertheless larger than that reported by the previously mentioned studies. Conversely, the studies in which significant risk factors were reported either did not specify whether the affected patients had chronic infections or disseminated disease, or included both types of patients. Consequently, it is difficult to specify whether the identified factors were predictive of chronic infection or of disseminated *S. stercoralis* disease.

Few studies have shown differences in eosinophilia between infected and uninfected subjects, although this parameter was found to be a significant indicator among infected patients. However, these series are of limited reliability because in some cases the presence of eosinophilia was a determinant factor for examining the stool samples of infected individuals, while other studies were conducted in patients with hematologic disease or in individuals reporting to a hospital for non-specified reasons and who may have had pathologic processes implying eosinophilia. In our study, based on a population of farm workers, the presence of eosinophilia was found to be a satisfactory indicator of infection, resulting in good sensitivity and excellent specificity. In addition, the good tolerability (side effects were detected in only 3%) to the treatment provided in our study contrasts with previously published data, with adverse effects reported in up to 30% of the cases.

Since the presence of eosinophilia was found to be the only indicator of *S. stercoralis* infection with high sensitivity and specificity, and the disease is prevalent among farm workers, with an important percentage of fatal complications and important economic costs, we believe that eosinophilia screening efforts should be made in our area in all farm workers with the aim of providing treatment without the need for stool examinations, regardless of the existing symptoms. In this context, treatment is inexpensive (3.4 Euros/person weighing an average of 70 kg), effective, and well tolerated in the chronic phase. In addition, screening for eosinophilia is less costly (1 Euro/person, net cost = 13,756 Euros) and more simple and reliable than the search for parasites in stools (42.5 Euros/person, net cost = 584,630 Euros). Accordingly, all subjects with eosinophilia > 400 cells/mm³ should receive treatment (net cost = 5,888 Euros). In this way, 2% of the infected individuals would be left untreated while 35% would be over-treated. The expected adverse reactions would affect the 3.1% who would then be subjected to stool examination (2,500 Euros).

We believe that this annual screening protocol, applied over a five-year period (maximum total cost = 93,579 Euros), together with an information campaign addressing the prevention and perpetuation of infection (approximately 100,000 Euros), could effectively eradicate the parasite from our geographic setting. In this way, a cost-effective reduction in the morbidity and mortality associated with *S. stercoralis* infection would be achieved. The theoretical mean number of infected individuals is 1,706, implying the possible existence of 222 severe forms of the disease (13%) and 68 deaths (30%) based on the data obtained from our series of 152 hospitalized patients, although these data cannot be extrapolated since the populations involved are different. The approximate global costs of hospitalization costs would reach 601,323 Euros.

In conclusion, although a study in the general population would be required, the high prevalence of *S. stercoralis* infection among farm workers in the area of Gandia, Valencia, Spain suggests that this region is an endemic zone for the disease. No parameter, other than eosinophilia, is suggestive of infection. Consequently, such epidemiologic information is essential for the diagnosis and treatment of this potentially fatal parasitosis. A program for preventing the severe forms of the disease, based on the treatment of risk groups with eosinophilia > 400 cells/mm³, would be highly profitable from the cost-benefit perspective in developed countries where the prevalence of other forms of parasitosis is low.

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