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Unsuspected strongyloidiasis in hospitalised elderly patients with and without eosinophilia

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ABSTRACT

The prevalence and associated factors of chronic uncomplicated strongyloidiasis were estimated among 200 consecutive elderly patients (aged ≥ 60 years) admitted to a general hospital in northern Italy. One-hundred patients had a peripheral eosinophil concentration ≥ 500 cells/μL (group A), and 100 were age- and gender-matched controls (group B). Measurements included serum IgG anti-*Strongyloides* antibody titre by an indirect immunofluorescence assay, combined with faecal culture for *Strongyloides stercoralis*. Anti-*Strongyloides* antibodies were detected in 28 patients (at high titre in 11 patients). Seropositivity was significantly more common among group A than among group B patients (OR 4.85). Strong seropositivity for anti-*Strongyloides* antibodies was associated with farm work ($p < 0.001$), but not with other patient characteristics or with signs and symptoms of strongyloidiasis. In conclusion, strongyloidiasis was relatively common among elderly in-patients; eosinophilia and a history of farm work were the most useful indications for this diagnosis.

Keywords Diagnosis, elderly patients, eosinophilia, occupational groups, serology, *Strongyloides stercoralis*

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INTRODUCTION

Strongyloidiasis is a parasitic disease caused by two species of intestinal nematodes, of which *Strongyloides stercoralis* is by far the most important clinically [1–3]. Infection by *S. stercoralis* usually results in chronic disease of the gut (uncomplicated infection), which can remain undetected for decades. The most common clinical findings reported in patients with uncomplicated infection include mild-to-moderate eosinophilia, skin lesions, gastrointestinal complaints (diarrhoea, abdominal pain) and respiratory symptoms (cough, asthma). Most of the literature concerning strongyloidiasis derives from retrospective reviews of the clinical records of patients, making it difficult to attribute a correct diagnostic value to each of these findings. Approximately one-third of patients are asymptomatic [4].

Under certain circumstances, parasite dissemination and/or hyper-infection may occur; this is often unrecognised and can be rapidly fatal. Typically, these complicated forms of strongyloidiasis occur following immunosuppression [5], often because of corticosteroid therapy or haematological malignancies, with the affected patients being elderly and hospitalised. According to recently issued guidelines [6], screening for asymptomatic strongyloidiasis is indicated for patients who have unexplained peripheral eosinophilia, or who have resided in or travelled to areas endemic for strongyloidiasis (i.e., tropical and sub-tropical regions of the world and the southern USA), even during the distant past. Patients who have never travelled to or resided in areas recognised as endemic are not considered to be at risk, although reliable and updated prevalence data are absent for many countries [7].

The aim of the present study was to investigate the seroprevalence of strongyloidiasis among

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elderly patients hospitalised in a rural area of northern Italy, and to verify prospectively the value of certain characteristics (e.g., eosinophilia, working history, signs and symptoms of strongyloidiasis) that might help to raise suspicion for a diagnosis of uncomplicated strongyloidiasis.

MATERIALS AND METHODS

Setting

The province of Novara in the north-west of Italy is an area of 1339 km^2, with 322 km^2 occupied by rice fields with an annual rice production of *c.* 200 000 tonnes. The climate is temperate, with a highest mean daily temperature between August 2002 and July 2003 of 31.4°C, a lowest temperature of 1.0°C, and annual rainfall of 800 mm. Novara Maggiore della Carità Hospital is a 717-bed acute-care general hospital serving a population of *c.* 344 000, of whom 26.4% are aged ≥60 years. The most recent epidemiological data, dating back to the late 1980s, indicated that strongyloidiasis was endemic in this area [8].

Patients

Cases and controls were identified prospectively from the computerised record system of the Laboratory of Clinical Pathology for 40 days during a 6-month period between 16 September 2002 and 17 March 2003. Search criteria were an eosinophil count $\geq 0.5 \times 10^9$/L, an age ≥60 years, and hospitalisation as an in-patient. Among 17 174 results scrutinised, 288 (1.68%) from 256 patients showed an eosinophil count $\geq 0.5 \times 10^9$/L. Of these, 156 were either outpatients, or patients who died or were discharged within 24 h of admission. The remaining 100 patients (group A) were contacted in their hospital ward on the same day and were asked to give informed consent to participate in the study and to answer a questionnaire administered by one of the authors (ES). The questionnaire requested details regarding each patient's employment history, and in particular, whether the patient had worked in farms and/or in rice fields, and the presence or absence of pruritus, skin lesions, recurrent abdominal pain and respiratory symptoms (chronic cough, asthma). Controls (group B) were 100 individually age- (± 2.5 years) and gender-matched patients admitted to the same hospital ward as the corresponding case, but who had an eosinophil count $< 0.5 \times 10^9$/L. Group B patients were also requested to give informed consent and to answer the same questionnaire. The study was conducted in strict accordance with the principles of the Helsinki Declaration.

Plasma samples for *S. stercoralis* serology were obtained by centrifugation of blood samples remaining after clinically indicated tests had been completed. Plasma aliquots were kept frozen at −30°C for ≤9 months before further processing.

S. stercoralis serology

IgG antibodies against *S. stercoralis* were detected by an indirect immunofluorescence assay developed and validated at the Centre for Tropical Diseases, Sacro Cuore Hospital, Negrar-Verona by two of the authors (MG, ZB). In brief, filariform larvae were isolated from fresh stool samples by the Baermann technique. The material obtained was washed five times in phosphate-buffered saline pH 7.2 at 4°C, exposed to cold acetone for 30 min, re-washed with phosphate-buffered saline, passed through a 14-mm filter and applied to glass coverslips. Study samples (25 μL), at an initial dilution of 1:20, were added to the coverslips in duplicate and incubated at 37°C for 30 min. After being rinsed twice with phosphate-buffered saline, the coverslips were incubated for 30 min at 37°C with 25 μL of 1:50 fluorescein isothiocyanate-conjugated anti-human IgG containing Evans blue 0.2% v/v (bioMérieux, Rome, Italy). After extensive washing, specimens were embedded in Fluoroprep (bioMérieux) and were viewed on a Leitz Laborlux S fluorescence microscope by an experienced microbiologist who was unaware of the clinical data.

The diagnostic value of the above assay was validated by testing blood samples from 122 patients with culture-proven *S. stercoralis* infestation and from 229 healthy blood donors. At sample dilutions >1:80, the sensitivity and specificity for a diagnosis of strongyloidiasis were 63% and 100%, respectively.

Faecal cultures

For each patient, three fresh stool specimens, obtained in the morning on different days and processed within 5 h, were plated on Mueller–Hinton agar and incubated at 26–30°C for ≥3 days. The culture plates were then evaluated by a single experienced microbiologist (CG). Samples in which either rhabditiform or filariform larvae, or channels carved into the agar by the parasite, were identified were considered to be positive.

Treatment with ivermectin (200 μg/kg in a single oral dose) was proposed for all culture-positive patients and, regardless of the culture result, for all patients with an indirect immunofluorescence test positive at a dilution >1:80.

Data analysis

Statistical analysis was performed with the BMDP New System v.2.0 software package (Statistical Solutions, Cork, Ireland). For continuous variables, distribution of data was checked for normality by Shapiro and Wilk's *W*-tests. When not departing significantly from the normal distribution, data were analysed by parametric methods (Student's *t*-test) and the variations observed among groups were calculated as means ± SD; otherwise, data were analysed by non-parametric methods (Mann–Whitney test; Spearman rank correlation coefficient) and the variations among groups were calculated as medians (range). Associations among categorical variables were analysed by Pearson's chi-square test and presented as observed frequencies and proportions. The probabilities of finding the outcome of interest (i.e., a positive test for anti-*Strongyloides* antibodies) were calculated in relationship to the characteristic defining cases and controls (i.e., the eosinophil count) and to several variables of interest as ORs (with 95% CIs calculated according to Woolf's logit-based formula) [9]. Mantel–Haenszel statistics were used to measure the common risk for both cases and controls of having positive anti-*Strongyloides* antibodies in the presence of a history of farm work. The confounding effect of multiple variables on the characteristic defining cases and controls (i.e., the eosinophil count) was calculated by stepwise logistic regression for a case-control study. The area under the receiver operating characteristic (ROC) curve was calculated to assess the

performance of the eosinophil count as a diagnostic test of strongyloidiasis in the study population [10]. For all tests, the level chosen to indicate statistical significance was $p < 0.05$ (two-tailed).

RESULTS

The study population comprised 118 male and 82 female patients, admitted to 22 different hospital wards. The two groups were well-matched in terms of demographical and biohumoral variables (Table 1). Skin lesions and respiratory symptoms were significantly more common among group A patients (Table 2). The skin lesions comprised urticaria (six group A and six group B patients), maculo-papular rash (three group A and three group B patients), erythema (diffuse: six group A and two group B patients; localised: five group A and five group B patients), eczema (five group A patients), localised pustules (two group A patients), herpes zoster (three group A and two group B patients) and other miscellaneous lesions (one erysipelas, one non-specific dermatitis and one vitiligo in group A; one purpura and one non-specific dermatitis in group B). None of the patients had skin manifestations pathognomonic of infection with *S. stercoralis* (e.g., larva currens).

Table 1. Selected demographical and clinical characteristics of the population studied

Characteristic	Group A (n = 100)	Group B (n = 100)	p
Female gender	41	41	1.000[a]
Age, years	74.3 ± 7.7	74.6 ± 7.6	0.774[b]
Body mass index, kg/m^2	24.9 ± 4.1	24.7 ± 4.0	0.737[b]
Farm work	47	40	0.318[a]
Work in rice fields	24	16	0.157[a]
Travel abroad	24	34	0.119[a]
BUN, mg/dL	18 (3–138)	19 (3–117)	0.585[c]
AST, U/L	20 (5–240)	23 (4–252)	0.181[c]
Eosinophil count, $\times 10^9$/L	0.7 (0.6–3.9)	0.1 (0.0–0.4)	< 0.001[c]

[a]Pearson chi-square test.
[b]Student's *t*-test.
[c]Mann–Whitney test.
BUN, blood urea nitrogen; AST, aspartate aminotransferase.

Table 2. Number of patients showing signs and symptoms compatible with strongyloidiasis in the population studied

Signs and symptoms	Group A (n = 100)	Group B (n = 100)	OR (95% CI)	p[a]
Pruritus	30	27	1.16 (0.63–2.14)	0.638
Skin lesions	33	20	1.97 (1.04–3.75)	0.037
Recurrent abdominal pain	35	46	0.63 (0.36–1.11)	0.113
Diarrhoea	29	19	1.74 (0.90–3.37)	0.098
Respiratory symptoms	47	13	5.93 (2.94–11.97)	< 0.001

[a]Pearson chi-square test.

Fig. 1 presents the scatter-plot of the individual titres of anti-*Strongyloides* antibodies in the two groups. Seropositivity for anti-*Strongyloides* antibodies at a titre >1:80 was found more often in group A (9/100) than in group B (2/100) patients (OR 4.85, 95% CI 1.02–23.05, p 0.030). A history of farm work was associated strongly with seropositivity for anti-*Strongyloides* antibodies; thus, among 87 patients who had worked on farms, either continuously or occasionally, 11 were seropositive. None of the remaining 113 patients with no history of farm work had anti-*Strongyloides* antibodies (OR not measurable; $p < 0.001$). The strength of this association was confirmed in both group A and group B patients (Mantel–Haenszel test, average risk 5.97, $p < 0.001$).

A correlation existed between the eosinophil count and the anti-*Strongyloides* titre (Spearman correlation coefficient, r 0.191, p 0.007). This correlation was caused entirely by the subgroup of patients with a history of farm work (n = 87, Spearman correlation coefficient, r 0.389, $p < 0.005$), and was not found with the remaining patients (n = 113, Spearman correlation coefficient, r − 0.043, p 0.649). The two seropositive patients in group B had an eosinophil count of 0.3×10^9/L, which was thee times the median value for this variable in group B. The area under the ROC curve of the eosinophil count for discriminating patients with an anti-*Strongyloides* titre >1:80 was 0.829, with an optimal cut-off value of 0.7×10^9/L.

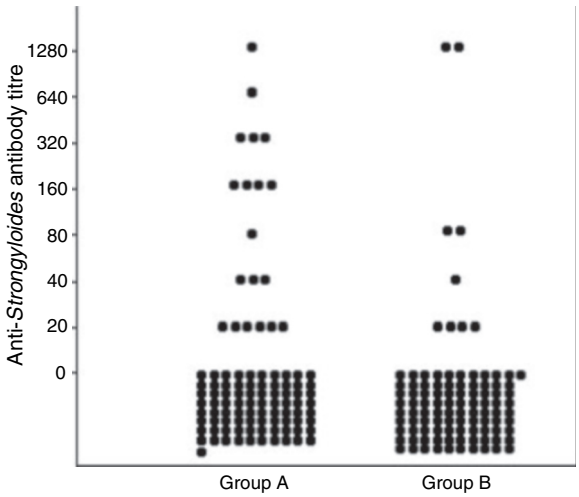

Fig. 1. Scatter-plot of individual anti-*Strongyloides* antibody titres in patients with elevated (group A) and normal (group B) peripheral eosinophil concentrations.

There was no significant association between seropositivity for anti-*Strongyloides* antibody and work in rice fields, a lengthy residence abroad, or the presence of pruritus, skin lesions, diarrhoea, recurrent abdominal pain or respiratory symptoms ($p > 0.10$ for each). Following multivariate analysis, the variables associated independently with group A (eosinophilia) were the presence of respiratory symptoms ($p < 0.001$), abdominal pain (p 0.021), seropositivity for anti-*Strongyloides* antibodies (p 0.031) and skin lesions (p 0.047) (goodness of fit chi-square = 94.8, p 0.514).

Within 6 months of the indirect immunofluorescence assay being performed, all 28 patients with a positive test, of any titre, were traced. Of these 28 patients, 13 had died, with the causes of death, deduced from the death certificates, being gastrointestinal tumours ($n = 2$), other tumours ($n = 4$), acute myocardial infarction ($n = 2$), heart failure ($n = 2$), stroke ($n = 2$) and aspiration pneumonia ($n = 1$). Among the 15 surviving patients, two had been diagnosed with strongyloidiasis by their physicians and had received appropriate treatment. One had diagnostic histology following gastrectomy, performed for causes unrelated to strongyloidiasis (gastric cancer); the other had been offered empirical anti-helminthic treatment by his physician, once notified of the serological test result, before stool cultures could be performed. The remaining 13 patients were invited to provide three fresh stool samples, on three separate days. Among the 39 specimens, three (8%) from two patients were positive. These two patients, as well as three other patients whose stool cultures were negative but who were serologically positive at titres ranging from 1:160 to 1:1280, were treated with oral ivermectin.

DISCUSSION

The present study revealed that the number of elderly in-patients living in a rural area in northern Italy with serological evidence of an immune response to *S. stercoralis* was substantial, reflecting the endemicity of the disease in this area [7,8]. When a patient has lived in or travelled to areas where *S. stercoralis* infection is endemic, and presents with compatible skin, respiratory or gastrointestinal symptoms [4], strongyloidiasis is an obvious diagnostic possibility. However, the parasite is capable of auto-infection of its host. A chronic well-regulated infection can remain virtually asymptomatic and be sustained almost indefinitely, or at least until host-cell-mediated immunity fails [11,12]. Therefore, a primary infection may have occurred up to six decades earlier, i.e., when living conditions in industrialised countries were much more similar to those in developing countries today.

To diagnose such cases, in the absence of updated epidemiological data, it is crucial to identify the patients at risk, and to establish how they should be tested. With regard to the first point, the present study indicated clearly that relying on symptoms in order to select a population with a high likelihood *a priori* of a positive test result would not constitute an effective strategy. In contrast, it suggests that addressing two simple questions (i.e., is there a history of farm work, and what is the eosinophil count?) may enable the clinician to limit the number of patients who need to be screened. The concept that a history of farm work and the presence of an elevated eosinophil count have value in the diagnosis of strongyloidiasis is not novel [13–16], but validation in a prospective, case-control study has not, to our knowledge, been performed previously. Farm work is thought to facilitate transmission of the parasite through direct contact with soil, in particular by working barefoot and by ingestion of contaminated non-potable water. It has also been known for many years that an elevation of the eosinophil count may alert the clinician to the possibility of parasitic disease. Eosinophilia is a relatively rare condition that, among outpatients, is related mostly to atopy [17], but is likely in the present setting to be the result of a Th2-type response to helminth antigens, causing increased production of eosinophil growth factors, particularly interleukin-5 [18,19]. As shown by the present data, eosinophilia among elderly in-patients can be attributed to respiratory and/or skin disorders, and is not indicative *per se* of strongyloidiasis. Moreover, the exact threshold level of the eosinophil count that should be used to define eosinophilia also remains undefined. The normal concentration of peripheral blood eosinophils is $0.015–0.65 \times 10^9$/L [20], with a diurnal variability [21], but a concentration of $0.5–1.5 \times 10^9$/L is considered by many to represent mild eosinophilia [22], whereas, for others, the upper limit of the normal range is 0.35×10^9/L [23]. White blood cell counts of elderly individuals do not differ from those of

young adults [24]. Nevertheless, it may help clinicians to know that, in the trade-off between sensitivity and specificity, the best cut-off value for considering a diagnosis of *S. stercoralis* infection on the basis of the eosinophil count would be 0.7×10^9/L.

Laboratory diagnosis of *S. stercoralis* infection is difficult to establish. Direct methods for isolation of the parasite are time-consuming, insensitive and costly, mainly because the intestinal worm load in uncomplicated infections is often very low and the output of larvae is minimal [2]. Thus, serological tests have been advocated because they are easier to perform and offer superior sensitivity [25]. However, in comparison with direct methods for parasite isolation, serological tests do not allow a distinction between past and current infection, and have inferior specificity because of cross-reactivity with other helminth infections. Nevertheless, where the prevalence of strongyloidiasis is supposedly high, the positive predictive value of serological tests is increased. Furthermore, although the indirect immunofluorescence test was very efficient in the present study, a standardised ELISA, with a reported sensitivity and specificity of >90%, may be preferred by others [26]. A cost-effective approach in endemic areas (including central, eastern and southern Europe) would be to use serological and cultural tests in sequence, i.e., to screen patients at risk with a serological test and, in the event of a positive result, to confirm the diagnosis by means of faecal cultures. However, since oral ivermectin, the treatment suggested currently for uncomplicated strongyloidiasis, is relatively inexpensive, effective and well-tolerated by most patients [27,28], it may also be reasonable to treat all patients with a high titre of anti-*Strongyloides* antibodies, regardless of the results of culture.

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