Strongyloidiasis: the Harada–Mori test revisited

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Human infection with the nematode *Strongyloides stercoralis* is endemic in some tropical and warm areas of the world, including parts of Europe and the southeastern USA [1]. The clinical presentation of strongyloidiasis is polymorphic, and the parasite may persist undetected for decades as a chronic, almost asymptomatic infection. Under immunosuppressive treatment, such as that administered after transplantation, extensive tissue invasion may develop, producing life-threatening systemic syndromes.

These may be easily prevented by treating patients before the immunosuppression is started. Therefore, it is essential to evaluate transplant candidates with a history of residence or travel in endemic zones, to rule out the possibility that they harbor *S. stercoralis*. Nevertheless, standard-concentration parasitologic methods may provide false-negative results, with the consequent risk of hyperinfection when immunosuppression begins. We describe such a case and recommend the Harada–Mori method for prospective screening of patients at risk.

CASE REPORT

A 47–year-old man was admitted as a candidate for heart transplantation because of end-stage ischemic heart disease. The infectious diseases specialist was consulted in March 1995 for evaluation of eosinophilia (16%; 2500/mm^3) found in the initial work-up.

The patient had lived in rural areas of Equatorial Guinea and Central America from 1979 to 1990. In 1990 he was evacuated to Spain because of serious bloody diarrhea, fever and eosinophilia (56%; 14300 eosinophils/mm^3). He was then diagnosed as having strongyloidiasis and adult *Ascaris lumbricoides* infestation, and was treated with three courses of thiabendazole, after which stool samples became negative for ova and parasites. No other parasites were found in stool, blood, skin snip biopsy or urine samples.

He remained in Spain after the treatment. His physical condition deteriorated, and he was admitted several times to different hospitals as a result of cardiovascular emergencies. Blood eosinophil counts were not reported to be high in any record available from this period of time.

On present admission, the patient complained of pruritus in the trunk and lower limbs and occasional appearance of a single, elongated pruritic rash on the waist and buttocks, which faded in a few hours. He did not have diarrhea. Physical examination disclosed scratching lesions on the waist.

Standard parasitologic studies, including examination of blood and skin for filariae, were all negative. Three stool samples concentrated by the formalin–ethyl acetate method and a string test were exhaustively examined for *S. stercoralis*, with negative results.

The Harada–Mori test was performed to increase the chances of detecting strongyloides infection. Approximately 6 g of freshly passed stools was smeared on six strips of blotting paper and placed in tubes containing a small amount of water. The tubes were incubated at 30°C in an upright position to allow the larvae to molt and migrate to the water. By the tenth day, a few filariform larvae of *S. stercoralis* were detected.

The patient received 2.5 g/day of thiabendazole for 5 days, with clinical and parasitologic cure. He also received three courses of ivermectin, even though the presence of *Strongyloides* has not been demonstrated again in subsequent stool samples. His follow-up has been unremarkable. His cardiac function is now stable, so heart transplantation has been postponed, but another course of ivermectin will eventually be provided before transplantation.

DISCUSSION

Strongyloidiasis is a widespread disease in warm, humid areas of the world. It is estimated that about 50 million to 100 million people are infected [2]. At the present time, autochthonous infection in Spain is extremely uncommon.

Humans usually acquire *S. stercoralis* infection by penetration of skin by the infective filariform larvae, usually after contact with contaminated soil. The worm

matures and multiplies within the human host, and may cause three possible clinical conditions: acute diarrhea, chronic silent or scarcely symptomatic gastrointestinal disease, and systemic infections (hyperinfection syndrome and disseminated strongyloidiasis) [3]. These last mentioned syndromes are usually associated with suppression of the host's immune response and in the case of solid organ recipients, symptoms develop in the first 6 months post-transplantation.

In the hyperinfection syndrome, overwhelming parasite load occurs, but the parasite is only found in the gastrointestinal and respiratory tracts. On the contrary, when disseminated strongyloidiasis occurs, other organs may be affected, with massive tissue invasion. Patients may present with cutaneous, gastrointestinal, central nervous system or pulmonary symptoms, and sustained or recurrent Gram-negative bacteremia or meningitis may develop due to the introduction of bowel bacteria, either along with penetration of infective larvae from the bowel or through damaged intestinal epithelium. Although eosinophilia is frequent, it may be absent in immunosuppressed patients. The mortality rate of the systemic syndromes may reach 77%, even when thiabendazole is provided [3–5].

The cutaneous manifestation of our patient is known as larva currens and occurs in association with autoinfection. It is an allergic reaction to filariform larvae that migrate under the skin, leaving linear urticarial papules. Two-thirds of individuals with chronic infection will develop transient and recurrent urticarial eruptions on the skin of the waist and buttocks.

A high index of suspicion is required to establish the diagnosis, and it is essential to consider *S. stercoralis* in immunosuppressed patients with eosinophilia, recurrent skin lesions, multiple pulmonary infiltrates, ileus with or without perforation, abdominal pain, malabsorption, meningitis or bacteremia. Chronic carriage is common among individuals environmentally exposed and has been found to persist for up to 30 years [6,7].

In conventional fresh smear preparations for ova and parasites, stool samples are concentrated by ethylacetate–formalin centrifugation, and one drop of resuspended fecal material is examined under a microscope. In chronic disease, a few rhabditiform larvae are passed in feces, and in such cases conventional methods have low sensitivity (27% for a single stool examination). In some reported cases it was necessary to submit up to eight samples or repeat wet mount examinations for a cumulative time of 10 work-hours before parasites could be demonstrated in a given patient [6], and, accordingly, some authors recommend the administration of pre-emptive therapy to all transplant candidates who have traveled to or resided in an area of endemic infection [8].

The Harada-Mori test-tube filter paper method increases the chances of recovering *Strongyloides* from intestinal samples [9]. This method utilizes the natural water tropism of larvae to concentrate them. A recent, unpreserved and unrefrigerated fecal sample is smeared on a strip of blotting paper. The strip is placed in a 40–mL screw-capped tube containing a few milliters of water which continuously soaks the paper. The tube is incubated at 24–28°C for up to 10 days. *Strongyloides* rhabditiform larvae migrate to the water and transform into filariform larvae. The water sediment is screened daily under a low magnification for living larvae, which should be differentiated from those of hookworm. This technique is simple, efficient, convenient, very easy to perform and may be requested from any clinical microbiology laboratory. Modifications of this method have been described. Among them, the Baermann technique uses larger volumes of feces, so increasing the sensitivity of the examination, which may become positive within hours [10].

Treatment of strongyloidiasis consists of thiabendazole or ivermectin. It is important to consider the possibility of relapses after therapy. Reported cure after six doses of thiabendazole (25 mg/kg per day, twice daily for 3 days) is 93%, but clinical or parasitologic failure after 6 months of follow-up may reach 33% [11].

Ivermectin is a new antiparasitic drug developed primarily for veterinary medicine that has proven efficacious in strongyloidiasis and other infections due to nematodes and ectoparasites [12–14]. Parasitologic cure was achieved in 24 out of 29 cases (83%) after a single dose of ivermectin (150–200 μg/kg). A recent comparative study indicated that ivermectin is as effective as thiabendazole for treating uncomplicated chronic strongyloidiasis and is associated with far fewer side effects [15].

The extensive use of cyclosporin has decreased the risk of hyperinfection syndrome in transplantation recipients, as this drug has some antiparasitic effect [16]. However, tacrolimus does not have anthelmintic activity, so when it is used as an immunosuppressant, both donor and recipient organs must be carefully examined for *S. stercoralis* [17].

We want to stress the importance of considering this infection in the preparatory work-up for transplantation, and recommend the Harada–Mori test to increase the sensitivity of the parasitologic screening.

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Staphylococcus aureus small colony variants: rate of selection and MIC values compared to wild-type strains, using ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin and moxifloxacin

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Small colony variants (SCVs) represent minority subpopulations of *Staphylococcus aureus* that grow slowly (>72 h) on routine media, yielding small, non-pigmented, non-hemolytic colonies. Although *S. aureus* SCVs have been recognized for many years [1–3], the connection between this phenotype and persistent recurrent infections has only recently been appreciated. Clinical and laboratory-generated *S. aureus* SCVs are frequently auxotrophic for menadione or hemin, two compounds required in the biosynthesis of menaquinone and cytochromes, components of the electron transport chain. The subsequent decrease in electron transport activity in SCVs may account for their resistance to a variety of antibiotics and other antistaphylococcal compounds, such as protamine, some antibiotics, and platelet microbiocidal proteins, and also provide a mechanism for their persistence within host tissues [4–6].

It is well known that *S. aureus* SCVs can be derived from clinical isolates both in vitro and in vivo following exposure to aminoglycosides and β-lactam antibiotics [1,2,4,5]. Mitsuyama et al. recently described the emergence of SCVs after exposure of wild-type parent strains to an MIC concentration of the fluoroquinolone pazufloxacin [7]. Emergent SCVs were half as susceptible to pazufloxacin and ciprofloxacin as wild-type *S. aureus*. Reduced susceptibilities of SCVs to these compounds were not a result of mutations in *gyrA*,