

Community-based seroprevalence survey of schistosomiasis and strongyloidiasis by means of Dried Blood Spot testing on Sub-Saharan migrants resettled in Italy

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SUMMARY

Serology is the most sensitive test for the diagnosis of schistosomiasis and strongyloidiasis, highly prevalent diseases in Sub-Saharan Africa (SSA). The collection of dried blood spots (DBS) on filter papers enables researchers to conduct community-based studies on this matter. We investigated the seroprevalence of schistosomiasis and strongyloidiasis on DBS in SSA migrants recently arrived in Italy. Seroprevalence was 10.2% for schistosomiasis and 2.7% for strongyloidiasis. The association between symptoms and positive serology was not statistically significant. Community-based serological screening of SSA migrants by means of DBS seems feasible and accepted; screening should be conducted independently of the presence of symptoms in such population.

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Schistosomiasis and strongyloidiasis are among the most prevalent infections in recently-arrived migrants from SSA, affecting around 10-20% of these subjects (Asundi *et al.*, 2019; Buonfrate *et al.*, 2018; Salas-Coronas *et al.*, 2018). Serologies are the most sensitive test for the diagnosis of both infections (Beltrame *et al.*, 2017; Requena-Mendez *et al.*, 2013) and are recommended by the majority of guidelines for screening in non-endemic countries (Centers for Diseases Control and Prevention, 2018). The majority of prevalence data on these two infections are based on screening carried out in specialized centers for tropical diseases (Asundi *et al.*, 2019; Buonfrate *et al.*, 2018; Salas-Coronas *et al.*, 2018) where a high level of diagnostic facilities, including availability of serological tests, are present. Data from community-based studies are lacking. Common obstacles to the use of serology in community-based epidemiological sur-

veys are storage and transportation of serum samples, particularly for studies in remote areas of the world. The collection of DBS from fingerpick on filter papers enables researchers to partially overcome these logistical problems (Sharma *et al.*, 2014), while minimizing the discomfort of blood sampling and the biological risk for the staff (Formenti *et al.*, 2016).

A few studies have compared the results of standard serology to the results of serology performed on DBS for the diagnosis of strongyloidiasis, finding good concordance and substantially equal sensitivity (Sharma *et al.*, 2014; Formenti *et al.*, 2016; Mounsey *et al.*, 2014; Tuailon *et al.*, 2020), while there are no data in the literature regarding serology performed on DBS testing for schistosomiasis. Hence, the use of serology on DBS could represent a streamlined way for enhancing strongyloidiasis and schistosomiasis testing, in particular in settings with limited resources.

In the present analysis, we investigated the seroprevalence of schistosomiasis and strongyloidiasis in a population of SSA migrants recently arrived in Italy and hosted in reception centers, using DBS collected from fingerpick.

The study was carried out from March 2018 to July 2019, involving migrants from SSA hosted in 13 re-

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ception centers, 12 for males only and 1 for males and females in five health districts of the provinces of Florence and Prato, Tuscany Region, Italy, and was proposed to approximately 700 SSA migrants. Only subjects aged >18 years, arrived in Italy less than three years before the enrollment date and never previously treated for any parasitosis were included. The study was approved by the ethics committee of Area Vasta Centro on 16/01/2018 (code 12298_bio). Migrants were enrolled in the reception centers during short information events on health topics (such as infectious diseases prevention) organized in the reception centers and delivered in English and French. Cultural mediators were involved when available. After proper explanation of the aims and methodology of the study, subjects willing to participate were asked to sign an informed consent, to answer a short questionnaire to collect epidemiological and clinical information, and provide a blood sample of 1 cm² on filter paper following puncture of the digital pulp. DBS were processed according to the procedure described in Supplementary information, a modified version of the procedure described by Formenti *et al.* (Formenti *et al.*, 2016) and analyzed with commercially available *Strongyloides ratti* ELISA (Bordier Affinity Products, Crissier, Switzerland) and *Schistosoma mansoni* Bordier ELISA (Bordier Affinity Products, Crissier, Switzerland). In order to evaluate the acceptability of the procedure, after the blood sample collection all subjects were asked if they would prefer this kind of test or phlebotomy.

A chi-square test was used to evaluate the association between dichotomous (presence of symptoms) and seropositivity. P values <0.05 were considered significant.

Even though the good performance of serological assays on DBS eluate for several conditions has already been proven in the scientific literature (Tuailon *et al.*, 2020), we evaluated the agreement of serological tests for schistosomiasis and strongyloidiasis used in this study on both DBS eluate and serum in a limited number of patient's samples. The agreement between the two alternative methods under study was assessed using Cohen's Kappa coefficient. The values of Cohen's Kappa coefficients were interpreted according to Landis and Koch: 1.00-0.81: excellent; 0.80-0.61: good; 0.60-0.41: moderate; 0.40-0.21: weak and 0.20-0.00: negligible agreement (Landis *et al.*, 1977).

Concerning the *Schistosoma mansoni* Bordier ELISA test, the agreement between DBS eluate and serum was evaluated in 20 patient's samples and resulted excellent (Cohen's Kappa=1, Table 1). Concerning the *Strongyloides ratti* ELISA test (Bordier Affinity Products, Crissier, Switzerland) the agreement between the two methods was evaluated in 20 patient's samples and resulted good (Cohen's Kappa=0.64, Table 2). Out of a population of 639 people hosted in the reception centers, 349 (55%) participated in health infor-

Table 1 - *Schistosoma mansoni* Bordier ELISA: the agreement between DBS eluate and serum.

	Serum	
	Positive	Negative
DBS eluate	8	0
	0	12
Cohen's Kappa	1	

Table 2 - *Strongyloides ratti* Bordier ELISA: the agreement between DBS eluate and serum.

	Serum	
	Positive	Negative
DBS eluate	1	0
	1	18
Cohen's Kappa	0.64	

mation meetings. Of these, 254 (73%) agreed to participate in the study. The rate of participation in the study among participants in the initial meeting was higher when cultural mediators participated in the information meeting. In detail, 35 of 37 (95%) decided to participate in the presence of a cultural mediator, while 196 of 312 (63%) participated when a cultural mediator was not available.

Among enrolled subjects, 97% were males (246/254) and 3% females (8/254). Median age was 25 years (range 18-49) and median residency time in Italy was 25 months (range 3-36). Participants came from 14 Sub-Saharan countries: the most frequent country of origin was Nigeria (109/254, 43%), followed by Guinea (29/254, 11%), Côte d'Ivoire (23/254, 9%) and Gambia (23/254, 9%). Ninety-two people (36%) were asymptomatic, while 162 (64%) reported one or more symptoms. The most frequent ones were cephalalgia (100/254, 39%), abdominal pain (84/254, 33%) and pruritus (59/254, 23%). Seroprevalence was 10.2% (26 out of 254) for schistosomiasis, 2.7% (7 out of 254) for strongyloidiasis. The association between symptoms and positive serology was not statistically significant.

All enrolled subjects were asked if they would prefer the performed procedure (puncture of the digital pulp) or phlebotomy and all reported to prefer the puncture of the digital pulp.

In this study we performed a community-based serological screening, using DBS, of SSA migrants resettled in Italy within the context of reception centers. The procedure was quite well accepted, since 73% of subjects to whom the procedure was presented voluntarily agreed to participate. All enrolled subjects reported to prefer the puncture of digital pulp compared to phlebotomy. It should be noted that this medical procedure was accepted even during the Ramadan period, during which some treatments are not accepted by religious people. Only two subjects re-

fused to perform due to religion prohibitions linked to Ramadan.

We found seroprevalences of 10.2% and 2.7%, respectively, for schistosomiasis and strongyloidiasis. All migrants with positive results were treated with a single dose of praziquantel 40 mg/kg for schistosomiasis and with a single dose of ivermectin 200 µg/kg for strongyloidiasis. Our findings showed significantly lower seroprevalences compared to those reported in a recent systematic review of the literature (24.1% and 14.6%, respectively, for schistosomiasis and strongyloidiasis). These differences may have three main explanations. First, the study setting: the majority of studies included in the systematic review of the literature (Asundi *et al.*, 2019) were healthcare center-based studies, which are prone to selection bias and which may lead to overestimation of the prevalence of the diseases, since symptomatic subjects are more likely to present to the clinic to be screened. Moreover, we used a technique (serology performed in DBS) which is known to be less sensitive compared to regular serological testing of serum samples, for example for strongyloidiasis (Formenti *et al.*, 2016). As far as we know, this is the first study that evaluates serology by DBS testing for the diagnosis of schistosomiasis. Moreover, the serological test used for schistosomiasis (*Schistosoma mansoni* Bordier ELISA, Bordier Affinity Products, Crissier, Switzerland) use *S. mansoni* antigens and has a decreased sensitivity for *S. haematobium*, which is the most prevalent species in Africa.

Our study demonstrates that symptoms which may be associated to strongyloidiasis and schistosomiasis, such as abdominal pain and itching, may not be used as criteria to select subjects for testing, since the presence of symptoms is not associated to seropositivity. This underlines the importance of screening independently of the presence of symptoms in such population. Recently, an economic analysis on different strategies for the management of strongyloidiasis in SSA migrants performed in the Italian setting showed that serological screening followed by treatment with ivermectin or universal presumptive treatment with the same drug are both cost-effective if compared to no treatment, highlighting that strongyloidiasis should no longer be neglected from the public health point of view in countries receiving migrants from endemic areas (Zammarchi *et al.*, 2020). So far, no similar studies exist for schistosomiasis; however, guidelines from the US, EU and Australia all recommend screening (or in some case presumptive treatment) for these two diseases for migrants from endemic countries (European Centre for Disease Prevention and Control 2018; Centers for Diseases Control and Prevention 2018; Chaves *et al.*, 2017).

In conclusion, community-based serological screening of SSA migrants by means of DBS seems to be feasible and accepted. This method could be used to

screen migrant subjects not only for strongyloidiasis and schistosomiasis, but also for other diseases such as HIV, HBV, and HCV by using capillary puncture outside healthcare facilities, with a simple and safe way of transportation (Tuailon *et al.*, 2020). The main problem linked to that procedure is the reduction of sensitivity which is expected for serology performed on DBS eluates, together with the current impossibility of automating the elution process, which still needs to be performed manually.

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