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Combination of Kato–Katz faecal examinations and ELISA to improve accuracy of diagnosis of intestinal schistosomiasis in a low-endemic setting in Brazil

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ABSTRACT

Considering the decrease of disease burden caused by intestinal schistosomiasis in many endemic settings, more sensitive diagnostic methods are needed to plan and monitor control measures. We conducted a cross-sectional survey in a rural community in northeast Brazil (317 inhabitants). A combined approach including repeated faecal examinations and ELISA testing was applied. In a first round, single stool samples were collected from 305 (96.2%) participants. Three Kato–Katz (KK) smears were prepared from each sample, and IgG ELISA was performed from serum samples. In the 85 cases of negative KK smears, but positive ELISA results, three additional faecal samples were collected in a second round, and another five KK smears prepared. In the first round of KK analysis, 11/287 (3.8%; 95% confidence interval; 1.92–6.75) were positive. After examining up to eight smears per individual (second round), prevalence of schistosomiasis increased to 8.7% (95% confidence interval: 5.9–12.5). In total, 96/287 (33.4%, 95% confidence interval: 28.0–39.2) samples were positive by ELISA testing. There were no false negative ELISA results. Specificity, positive and negative predictive values of ELISA as compared to up to eight KK smears from three stool samples (reference diagnosis) were 72.9%, 26.0% and 100%, respectively. A single KK smear detected only 12% of the 25 infections; this increased to 44% (three smears, one stool sample), 84% (five smears, three stool samples) and 96% (six smears, four stool samples). We conclude that in low-endemic areas in Brazil the use of KK continues being an important tool. The additional benefit of preparing more than six KK smears from repeated stool samples is negligible. ELISA may be useful for screening populations, with subsequent confirmation of diagnosis by KK or other more sensitive, but highly specific methods.

1. Introduction

In the last years, both prevalence of intestinal schistosomiasis and morbidity caused by *Schistosoma mansoni* have been reduced significantly in Brazil, but transmission continues in many regions (Enk et al., 2008a). In the northeast of the country, some isolated foci of intestinal schistosomiasis remain, with low intensity of transmission and consequently low parasite burden. In Ceará State, the intermediate host *Biomphalaria straminea* occurs in these well-defined foci. Prevalence is highest in children and women who swim in the rivers and creeks, or wash clothes.

The Kato–Katz (KK) technique (Katz et al., 1972) is recommended by the World Health Organization as standard diagnostic method of intestinal schistosomiasis, as it is simple and of low cost, and the intensity of infection can be estimated (Teles et al., 2003). However, KK smears and other parasitological methods frequently fail to detect infections of low intensity, mainly due to day-to-day variation of egg excretion (Barreto et al., 1990; De Vlass and Gryssels, 1992; Engels et al., 1996; Kongs et al., 2001). Thus, more sensitive tests such as serological diagnosis or PCR could be a useful complement or in future even substitute parasitological tests (Mott and Dixon, 1982; Ruppel et al., 1990; Gui et al., 1991; Doenhoff et al., 1993, 2004; Hamilton et al., 1998; Rabello et al., 2002). The so-called FLOTAC technique uses a larger amount of stool for the microscopic detection of eggs than KK and thus sensitivity is increased, especially in case of low infection intensity (Knopp et al., 2009). However, these methods are more labour and cost intensive.

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The Brazilian Schistosomiasis Control Program relies on selected treatment with praziquantel of individuals with positive KK tests, rather than general mass treatment, which further emphasizes the need for more sensitive methods that can be used in field settings (Santana et al., 1996). Individuals with low intensity of infection would not receive specific treatment, limiting the impact of community control efforts (Noya et al., 2007). In the present study, we performed repeated faecal examinations and ELISA testing of serum samples to improve accuracy of diagnosis of intestinal schistosomiasis in a low-endemic setting in northeast Brazil.

2. Materials and methods

2.1. Ethical aspects

The study was approved by the Ethical Review Board of the University Hospital of the Federal University of Ceará (Brazil). Informed written consent was obtained from study participants or in the case of minors from their guardians. In case of *S. mansoni* infection, a single dose of praziquantel (40 mg/kg body weight) was given. Participants with positive faecal examination for intestinal helminths were treated with albendazole (400 mg, single dose).

2.2. Study area, population and design

We conducted a cross-sectional survey in the rural community "Caititú de Cima" in Pacoti municipality in Ceará State (northeast Brazil). Pacoti (population 11,500) lies in the Baturité Mountains (altitude 736 m) about 130 km from Fortaleza, the State capital.

In 2002, 23% of the population of the municipality lived in the urban centre, and 77% in small hamlets scattered in the mountains. Cultivation of fruits and vegetables, which are sold on the markets of Fortaleza, forms the main occupation in the hamlets. Mostly, many perennial creeks and small rivers running down the mountains support populations of the intermediate host *B. straminea* (Oliveira et al., 2006). In the study area, the State's schistosomiasis control program has been realizing KK stool examinations and selective treatment of positive individuals with praziquantel for more than 30 years.

At the time of the survey (2007/2008), Caititú de Cima consisted of 88 households with 317 inhabitants. Twelve infants (<2 years of age) were excluded from the study, resulting in a target population of 305 individuals.

Community meetings were held to explain the objectives of the study. Each household in the community was visited. Family members present at this occasion were interviewed, using pre-tested structured questionnaires.

2.3. Stool examinations and ELISA

For Kato–Katz stool examinations, labelled plastic containers were distributed for collection of faecal samples. On the following day, the containers were collected and sent to the local health care centre for parasitological examination. Stool samples were prepared in a field laboratory on the same day. To identify intestinal helminths and *S. mansoni* eggs, approximately 50 mg faeces were prepared by the KK method (Katz et al., 1972). Intensity of infection, as expressed by eggs per gram (epg) of faeces, was calculated by multiplying each slide count by 20.

At this first round of faecal examination, three KK smears were prepared from each sample.

For performance of ELISA testing, venous blood was collected using a commercially available system (Becton Dickinson Vacutainer Systems, Franklin Lakes, USA). To detect *S. mansoni* IgG antibodies from serum samples, we used soluble egg antigens of *S. mansoni* (SEA) and performed ELISA testing according to standard procedures (Colley et al., 1977; Engvall and Perlman, 1971). The cut-off was defined based on the mean value of the optical density ± 2 standard deviations in a hospital-based population ($n = 32$) from the city of Fortaleza, with negative parasitological faecal examination.

In order to reduce disturbance of the study population, we did not repeat faecal examinations in the case of positive KK results. In the case of negative KK results and positive ELISA, in a second round, another three faecal samples were collected on three consecutive days. In total, five KK smears were prepared from these three additional samples (one smear from the first, one from the second, and three from the third sample).

2.4. Data entry and statistical analysis

Data were entered using an Excel® spreadsheet and checked for entry-related errors, by comparing data entries with original data forms. Then data were transferred to Stata® software package (version 9.0; Stata Corporation, College Station, USA) for analysis.

Specificity and negative predictive value of IgG ELISA and the respective confidence intervals were calculated using the combined results of all eight KK smears as reference diagnosis ("gold standard"). As additional faecal samples were only collected when KK smears were negative and ELISA tests positive in the first round, calculation of sensitivity and positive predictive value were calculated based only on the first round of faecal examinations (three KK smears).

To reduce inter-observer variation, all slides were read by a single investigator. To perform quality control, 10% of slides were randomly selected for cross-reading by a reference microscopist who was blinded to the results of the first reading. Divergence was less than 5%.

3. Results

3.1. Parasitological examinations

In the first round of parasitological analysis, both faecal and blood samples were available from 287/305 (94.1%) inhabitants. Of these, 155 (54%) were males and 132 (46%) were females; mean age was 32.5 years (standard deviation: 21.3 years).

Eleven (3.8%; 95% confidence interval; 1.92–6.75) were positive for *S. mansoni* eggs: three after reading the first slide (estimated prevalence 1.0%), five at the second slide (2.8%) and three at the third slide (3.8%). In total, 96/287 blood samples (33.4%, 95% confidence interval: 28.0–39.2) were positive for *S. mansoni* antibodies. In addition, the following intestinal helminths eggs were detected: *Trichuris trichiura* 25 (8.7%); *Ascaris lumbricoides* 19 (6.6%), and hookworms 15 (5.2%).

Eighty-five individuals were ELISA-positive, but had three negative KK smears. Of these, 56 (66%) provided at least one additional faecal sample. In the first of these faecal samples, an additional 5/56 (8.9%) were positive, in the second 5/51 (9.8%), and in the third 4/46 (8.7%), totalizing 14 infected individuals detected in the second round.

Thus, combining all KK smears (reference diagnosis), schistosome eggs were found in 25/287 individuals (prevalence: 8.7%; 95% confidence interval: 5.9–12.5). Of these, 13 (52%) were males and 12 (48%) were females. Mean age was 27.6 (standard deviation: 16.8 years). In all cases, intensity of infection was low (<50 epg).

3.2. Diagnostic performance

Fig. 1 shows the cumulative fraction of positive KK smears, according to the number of slides examined (sensitivity). With examination of one smear, only 12% of the 25 infected individuals were detected. This increased to 44% (three smears, one stool

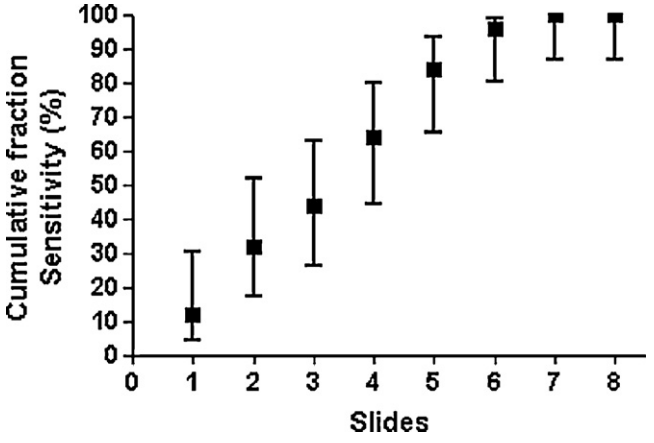

Fig. 1. Cumulative fraction (sensitivity) of KK smears, according to the number of slides examined, as compared to eight KK smears as reference diagnosis ($n = 25$). Slides 1–3: first round of faecal examinations (one stool sample); slides 4–8: second round of faecal examinations (up to three stool samples). Vertical bars indicate 95% confidence intervals.

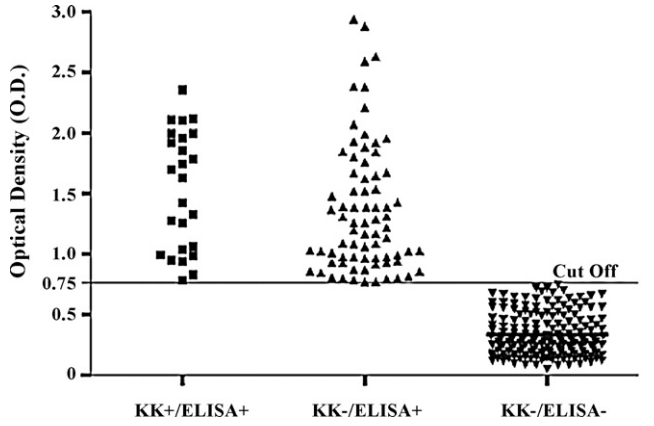

Fig. 2. Distribution of individuals according to results of faecal examinations (KK) and ELISA tests ($n = 287$). In all individuals with negative ELISA test, KK was negative.

sample), 84% (five smears, three stool samples) and 96% (six smears, four stool samples). The additional benefit of reading more than six smears (four stool samples) was low (Fig. 1).

Fig. 2 details results of individuals, according to ELISA and KK results. There were no false negative ELISA results, and ELISA was positive in all cases when eggs were detected in KK smears. Of the 42 individuals with positive ELISA serum samples and negative faecal samples, 22 (52.3%) stated in the interview that they never had had schistosomiasis and never had received specific treatment.

Diagnostic performance of ELISA as compared to KK as the reference diagnosis is depicted in Table 1. The negative predictive value of ELISA was high, but specificity and positive predictive values were low.

Table 1
Diagnostic performance of IgG ELISA based on results of eight KK slides (four stool samples) as reference. Calculations of sensitivity and negative predictive value are based on three KK smears of the first round of faecal examinations, as subsequent faecal examinations were only done in the case of positive ELISA results—see Section 2.

	% (95% confidence interval)
Sensitivity	100 (67.9–100)
Specificity	72.9 (67.0–78.1)
Positive predictive value	26.0 (17.9–36.2)
Negative predictive value	100 (97.5–100)

4. Discussion

Since 1976, parasitological stool examination has been used by the Brazilian state schistosomiasis control programs as the criterion for selective praziquantel treatment (Gargioni et al., 2008). Our data confirm that in a low-endemic setting in northeast Brazil faecal examination has still an important value to screen populations and to monitor ongoing control efforts, but that in addition more sensitive methods are useful.

Sensitivity of Kato–Katz (KK) technique is usually low, and using a single KK smear considerably underestimates prevalence of schistosomiasis, principally in low-endemic settings. By increasing the number of KK smears per sample and the number of faecal samples performed, we increased sensitivity considerably. Other investigators from Southeast Brazil suggested previously increasing the number of stool samples, and the number of smears per sample, in low-endemic areas, to achieve higher sensibility of diagnosis (Gonçalves et al., 2006; Enk et al., 2008a,b). Enk et al. (2008b) observed an increase in estimated prevalence from 13.8% (one KK smear) to 19.0% (six KK smears), using a single stool sample. The increase of the number of stool samples resulted in an estimated prevalence of 27.2% (10 smears from three stool samples) (Enk et al., 2008a,b). Lin et al. (2008b) reported also a considerable benefit of repeated stool examinations: prevalence of schistosomiasis caused by *Schistosoma japonicum* increased from 8.3% (one KK smear) to 12.7% (three smears) and 18.4% (six smears) from two stool samples. Similar to our data, increase of prevalence after the sixth KK slide was low, and the additional resources needed did not justify using more than six smears.

Previous to our study, Berhe et al. (2004) used a similar approach in a high endemic setting in Ethiopia: repeated KK smears were performed from a single stool sample, and in case of negative results, an additional stool sample was collected and three KK smears were examined. Prevalence from a single faecal sample increased from 31.3% (one KK smear) to 45.7% (two smears) and 52.1% (three smears). However, different to the low-endemic setting of our study, estimated prevalence did not increase considerably with an additional stool sample collected and three more KK smears examined.

Consecutive faecal examinations may pose logistical problems and increase costs (Van Lieshout et al., 2000). This would also mean an increased workload of field personnel. As a consequence of reduced transmission in the last years and a reduced number of endemic communities, the number of individuals examined has been reduced steadily in Ceará State—the still existing infrastructure and human resources may be used to increase the number of slides read per individual, without considerable additional financial or logistical input from the health sector.

In Brazil, the control program would benefit from inclusion of more sensitive serological diagnostic methods to screen populations in the mostly low-endemic areas. Sensitivity of ELISA was high in our study and has been described to be very high previously, in both low and high prevalence settings. For example, in China sensitivity of IgG ELISA for *S. japonicum* ranged from 79.3% to 87.4%, but specificity was low (38.9–53.5%), as compared to KK (six smears examined) (Lin et al., 2008a). Similar to these results, in our study specificity of ELISA was low. ELISA testing requires laboratory facilities, trained personnel, and more financial resources and should be first applied in sentinel areas and populations to monitor ongoing transmission and elimination efforts.

Selective repetitive faecal examinations may be applied in ELISA-positive individuals for confirmation of diagnosis. In fact, ELISA testing in the population with consecutive repetitive KK faecal examinations is a feasible approach for screening and control programs in low-endemic settings. A similar approach has been done in China by Zhou et al. (2007) who screened a population of

more than 290,000 by ELISA. In case of positive ELISA, three KK smears were examined, but from a single stool sample. Only 9% of ELISA-positive individuals had positive KK smears. In a similar way, Lin et al. (2008a) proposed using IgG ELISA as a screening tool in the entire population, and consequently testing only positive ELISA results by repeated KK examination, and consecutive selective treatment.

Our study is subject to limitations. First, we only repeated collection of stool samples in the case of positive ELISA tests, and subjects with negative KK and negative ELISA in the first round were not further investigated. On the other hand, our data have shown that additional stool samples might detect more cases of schistosomiasis. Thus, we cannot exclude that there might have been false negative ELISA results in one or the other case, and interpretation of 100% sensitivity of ELISA test should be taken with care. In addition, a considerable number of individuals did not provide additional stool samples in the case of positive ELISA tests, but negative KK smears in the first round. Thus, we may have missed some infected individuals and underestimated the prevalence of schistosomiasis in the area to some degree. Finally, the insignificant increase of sensibility from the sixth to seventh and eight KK smear (fourth stool sample) may be partially caused by day-to-day variation of eggs in stool, as observed previously (Engels et al., 1996; Kongs et al., 2001; Zhang et al., 2009).

We conclude that in areas with low resources, repeated KK examinations continue to be an option for control programs. ELISA is useful as a population-based screening method, with consecutive confirmation of diagnosis by parasitological methods. This will help reducing workload of KK smear examinations. The diagnostic potential and applicability of other more sensitive and possibly more specific methods, such as PCR and FLOTAC, need to be assessed systematically in future studies (Rabello et al., 2002; Knopp et al., 2009; Xia et al., 2009).

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