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Mapping of *Schistosoma mansoni* in the Nile Delta, Egypt: Assessment of the prevalence by the circulating cathodic antigen urine assay

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ABSTRACT

In line with WHO recommendations on elimination of schistosomiasis, accurate identification of all areas of residual transmission is a key step to design and implement measures aimed at interrupting transmission in low-endemic settings. To this purpose, we assessed the prevalence of active *S. mansoni* infection in five pilot governorates in the Nile Delta of Egypt by examining schoolchildren (6–15 years) using the Urine-Circulating Cathodic Antigen (Urine-CCA) cassette test; we also carried out the standard Kato-Katz (KK) thick smear, the monitoring and evaluation tool employed by Egypt's national schistosomiasis control programme. Prevalence rates determined by the Urine-CCA test for all governorates were higher than those determined by KK ($p < 0.01$). Of 35 districts surveyed in the five governorates, *S. mansoni* infection was detected in 19 districts (54.3%) using KK, and in 31 districts (88.6%) by Urine-CCA ($\chi 2 = 9.94$; $P = 0.0016$). *S. mansoni* infections were detected by Urine-CCA, but not by KK in 12 districts (34.3%), and infection was not detected by either of the two diagnostic methods in four districts in Qalyubia governorate. Males and higher age-groups have significantly higher Urine-CCA prevalence rates. Based on the findings of the current S. mansoni mapping exercise, authorities of the Ministry of Health and Population (MoHP) adopted a new elimination strategy by readjusting thresholds for mass treatment with praziquantel and targeting all transmission areas. MoHP is now planning to remap in all other endemic governorates using Urine-CCA with the aim of identifying all areas of transmission where the elimination strategy should be applied.

1. Introduction

Two forms of schistosomiasis (intestinal caused by *S. mansoni* infection and urogenital by *S. haematobium* infection), have been endemic in Egypt since ancient times. *S. haematobium* infection prevails along the Nile Valley south of Cairo, whereas *S. mansoni* infection is restricted to the Nile Delta. The National Schistosomiasis Control Programme (NSCP), established in 1977, has been successful in significantly decreasing the prevalence of the two forms (El Khoby et al., 2000).

Prevalence of *S. mansoni*, as assessed by a single Kato-Katz thick smear, had consistently decreased among the general population in the Delta from 14.8% in 1993–2.7% in 2002, and further declined to 1.5% in 2006, due to application of intensive control measures (WHO, 2007; WHO, 2011). Also, the intensity of infection, measured by egg count, has decreased considerably. However, there were still "hot spot" transmission foci with prevalence rates of about 10% in 2006. The number of the "hot spots" was 136 in 2010 and decreased to 88 in 2013, of which 83 were in Lower Egypt (Nile delta governorates) and 5 in Upper Egypt (Barakat, 2013). Currently, *S. mansoni* endemic areas can be grouped in three categories: areas with prevalence of ≥3%, areas with prevalence of <3% and those

Abbreviations: CCA, circulating cathodic antigen; CIs, confidence intervals; KK, Kato-Katz; MDA, mass drug administration; MoHP, Ministry of Health and Population; NSCP, National Schistosomiasis Control Programme; WHO, World Health Organization.

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with no autochthonous cases. Thus, most of the endemic areas are currently of low transmission and hence the Ministry of Health and Population (MoHP) aims for elimination of the infection (reducing incidence of infection to zero), in line with World Health Assembly Resolution 65.21 (2012; "Elimination of schistosomiasis"), and the recent World Health Organization (WHO) strategy (WHO, 2009, 2013).

The current *S. mansoni* epidemiological data are based on detection of schistosome eggs using a single Kato-Katz (KK) thick smear, the standard monitoring and evaluation tool employed by the MoHP's NSCP. However, the sensitivity of a single examination, especially in areas of low transmission, can be very low due to a combination of factors. These include variation in the distribution of eggs within a single stool specimen, day-to-day variations in egg excretion and random distribution effects (Hall, 1981; De Vlas et al., 1992; Engels et al., 1996; Engels et al., 1997; Kongs et al., 2001; Utzinger et al., 2001). The sensitivity of KK can be improved by examination of multiple samples, but this is impractical for field work. Recently, a cassette format test for active *S. mansoni* infection, based on detection of adult worms' circulating cathodic antigens (CCA) in urine samples, has been developed, evaluated, and is currently commercially available (Shane et al., 2011; Coulibaly et al., 2011; Tchuem Tchuenteí et al., 2012; Colley et al., 2013). Previous studies in African countries indicated that the Urine-CCA test is more sensitive than triplicate KK examinations in areas of low endemicity (i.e. low prevalence and intensity of infection) (Coulibaly et al., 2011). The present study aimed at more accurately mapping the distribution of intestinal schistosomiasis in five Nile Delta governorates as a first step towards extensive remapping in all *S. mansoni* endemic areas, in the framework of Egypt's efforts to achieve transmission elimination. In particular it assessed the prevalence of *S. mansoni* infection using the Urine-CCA test in schoolchildren, compared it with data obtained by the standard KK thick smear, and contributed to revision and updating of the MoHP's mass treatment policy.

2. Material and methods

2.1. Ethics statement

The present work was conducted as a standard and regular part of the MoHP's NTD Programme of public health fieldwork to evaluate the status of intestinal schistosomiasis in schoolchildren in anticipation of moving from morbidity control to elimination (WHO, 2013). The Ethics Review Committee of the Faculty of Medicine, Ain Shams University reviewed and approved the study protocol. The work included only non-invasive collections of stool and urine specimens and their examination by assays of two different sensitivities in order to obtain population data needed for programmatic decisions concerning elimination efforts. Thus, individual consent was not obtained, but rather consent was obtained at the school and community administrative levels for this public health programme.

2.2. Study population

The present work was carried out in five Nile Delta governorates (i.e. Behira, Dakahlia, Kafr El Sheikh, Qalyubia and Sharqia governorate), during March–April 2016. A convenience sampling methodology was employed to maximise the probability of identifying endemic settings. In each governorate a number of schools in rural areas of a study district were sampled, with 100 children selected per school. [3] Names of participating children were not recorded; they were anonymously coded according to their number of the classroom list. Based on previous data from Kato-Katz (KK) stool examinations, three endemicity settings were selected: (1) areas reporting *S. mansoni* prevalence of ≥3%; (2) areas with prevalence of <3% and more than zero percent, in the last school assessment (last scholastic year 2014–2015); and (3) areas reporting zero prevalence during the last three years. In each category, schoolchildren, 6–15 years of age, were examined.

The last annual schistosomiasis treatment, prior to this work, was implemented during the 2014–2015 scholastic year. The MoHP strategy calls for implementing targeted treatment of schoolchildren using praziquantel (PZQ) in each school where prevalence in a sample of its population is ≥2%. Moreover, the entire population aged ≥3 years of a village or satellite village (Ezba or sub-village, average population 1000) is treated (i.e. mass drug administration, MDA) when community prevalence, assessed in ≥100 of its inhabitants, is ≥3%.

2.3. Stool examination

Field assistants collected stool samples from schoolchildren enrolled in the study. Well trained laboratory technicians prepared and examined stool samples using a single KK thick-smear. The results were reported as negative/positive. Approximately 10% of the KK negative slides were reexamined by a senior laboratory technician. Eggs of soil transmitted helminths (STH; *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) were also reported.

2.4. Urine collection and urine-CCA diagnosis

The WHO Representative Office, in Cairo, supplied MoHP with 15,000 Urine-CCA cassette tests (Rapid Medical Diagnostics, Pretoria, South Africa; batch number 50174). Urine samples for the CCA test and stool samples for KK examination were collected from children in target schools. Urine samples were transferred to the district laboratory and processed within 2–3 h from collection. The Urine-CCA test was performed according to the manufacturer's instruction. Briefly, one drop of urine was added to the sample well of the cassette and allowed to absorb. Then, one drop of test buffer, provided with the kit, was added to the well and the assay was allowed to develop. The test was read 20 min after the buffer was added. Any line in the test area was considered positive. The test was considered invalid if the line developed after 25 min after the buffer was added or no control line was developed. The test was read and agreed upon by two observers (laboratory assistant or lab technician), and in case of disagreement results were discussed with a senior lab technician. Positive colour reactions were compared to the colour of the control line and arbitrarily scored as trace (faint-very faint band), weak (+), medium (++) and strong (+++).

The original study protocol called for testing only KK negative subjects by the Urine-CCA test. However, because the number of KK positives was expected to be small and to allow laboratory technicians to see intense positive bands, urine samples from all children examined by KK were also tested by the Urine-CCA.

2.5. Statistical analysis

Data were checked for its completeness and consistency. Data entry was done on Microsoft Excel database spreadsheet. Qualitative data was summarised by frequencies and percentages. Chi-square test was used in the analysis. Confidence intervals and odds ratio were calculated with STATA 10 Programme. The rest of the analysis was done with SPSS programme version 13.0. Non-overlapping 95% confidence intervals (CI) or a "P value" of less than 0.05 was considered statistically significant.

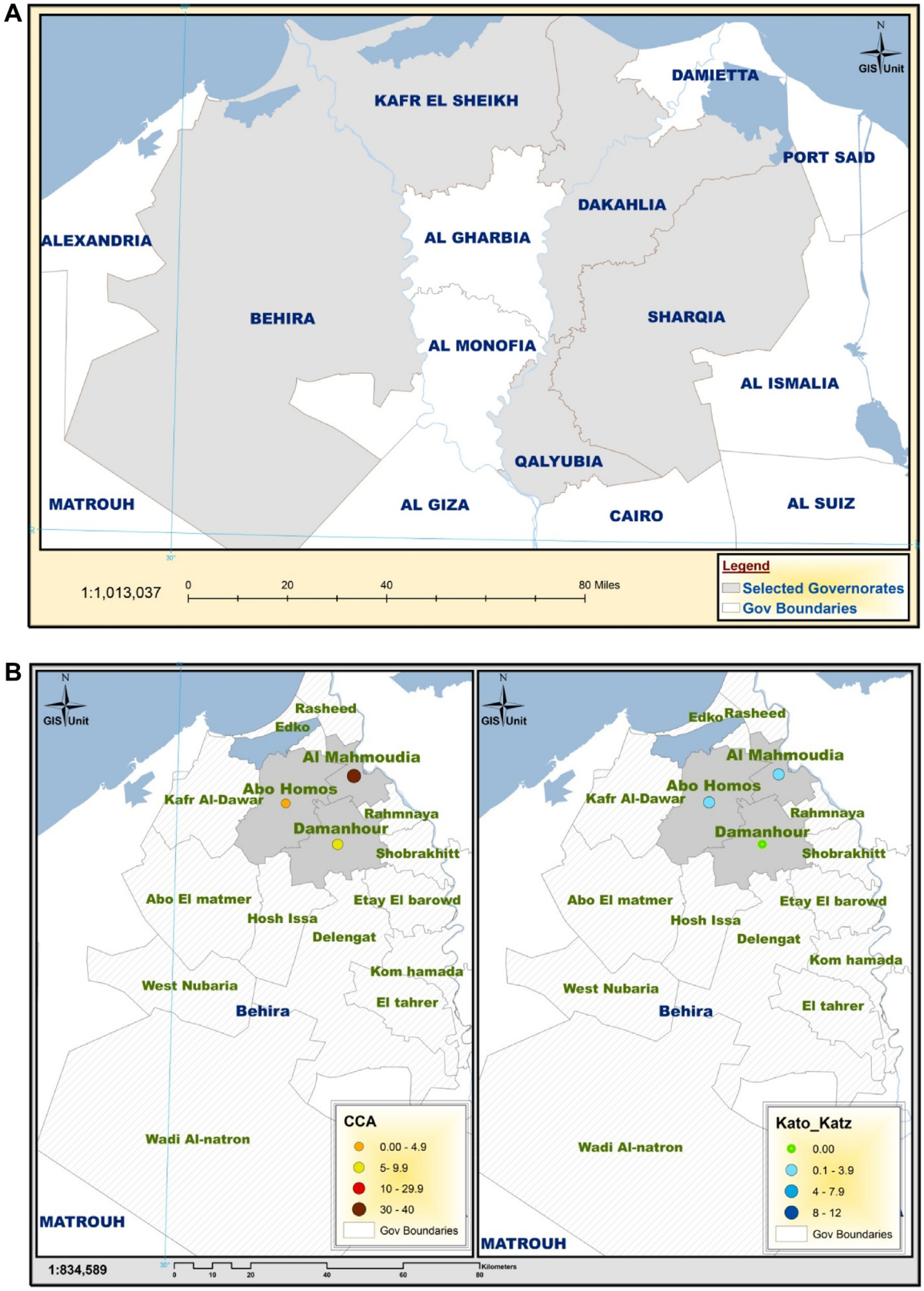
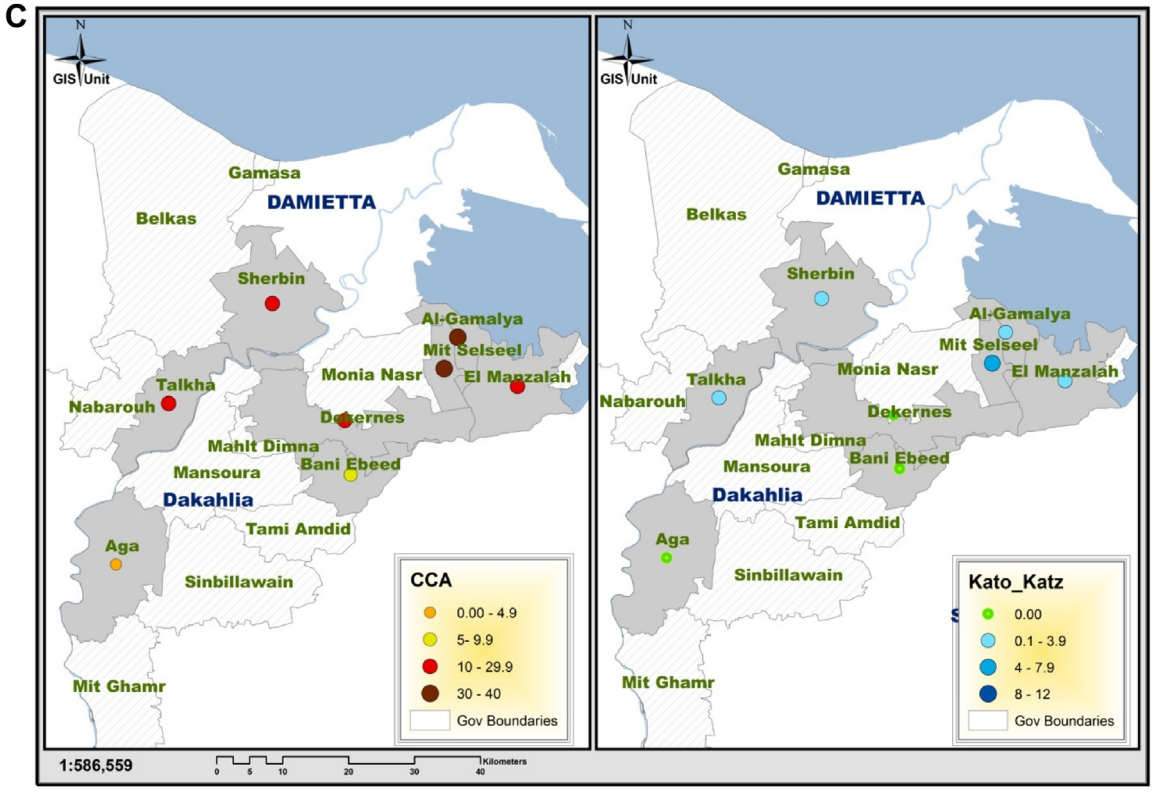
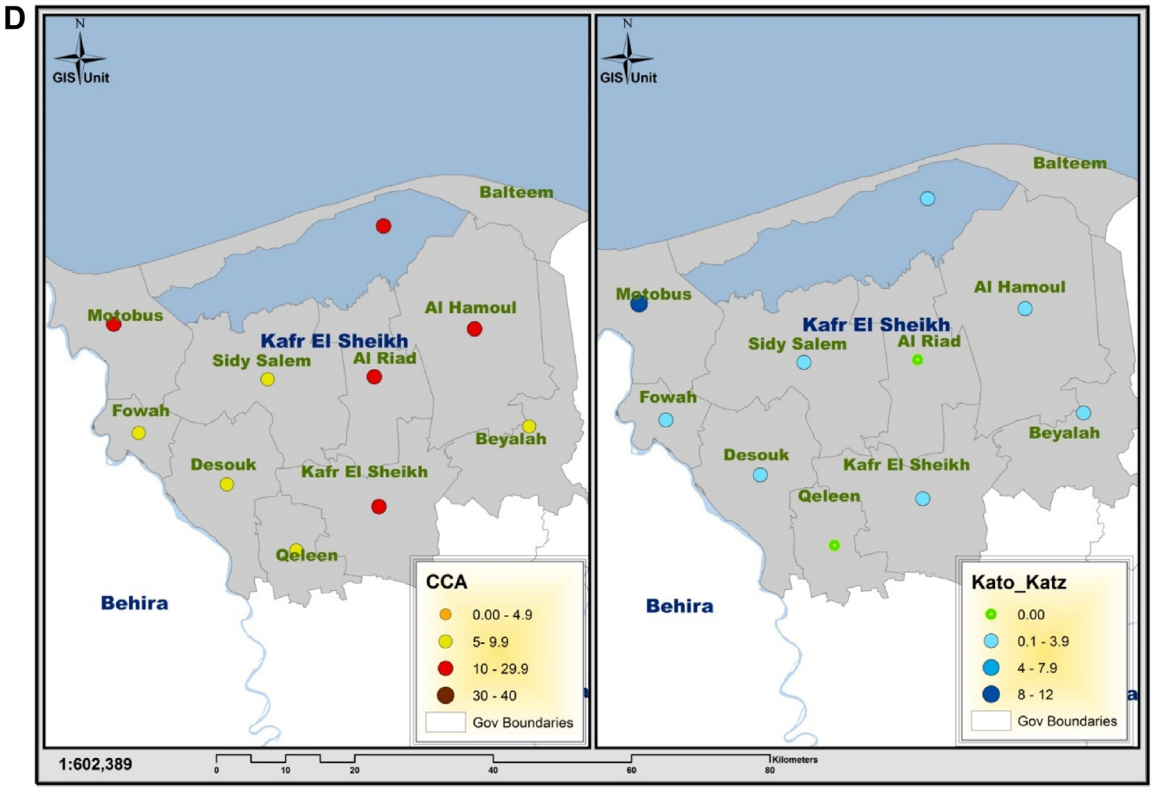


Fig 1. Maps of survey sites (governorates and districts). A. Map of the Nile Delta showing location of study governorates. B. Map of Behira showing the prevalence of *S. mansoni* infection at each district as determined by Kato-Katz and Urine-CCA techniques. C. Map of Dakahlia showing the prevalence of *S. mansoni* infection at each district as determined by Kato-Katz and Urine-CCA techniques. D. Map of Kafr El Sheikh showing the prevalence of *S. mansoni* infection at each district as determined by Kato-Katz and Urine-CCA techniques. E. Map of Qalyubia showing the prevalence of *S. mansoni* infection at each district as determined by Kato-Katz and Urine-CCA techniques. F. Map of Sharqia governorate, showing the prevalence of *S. mansoni* infection at each district as determined by Kato-Katz and Urine-CCA techniques.





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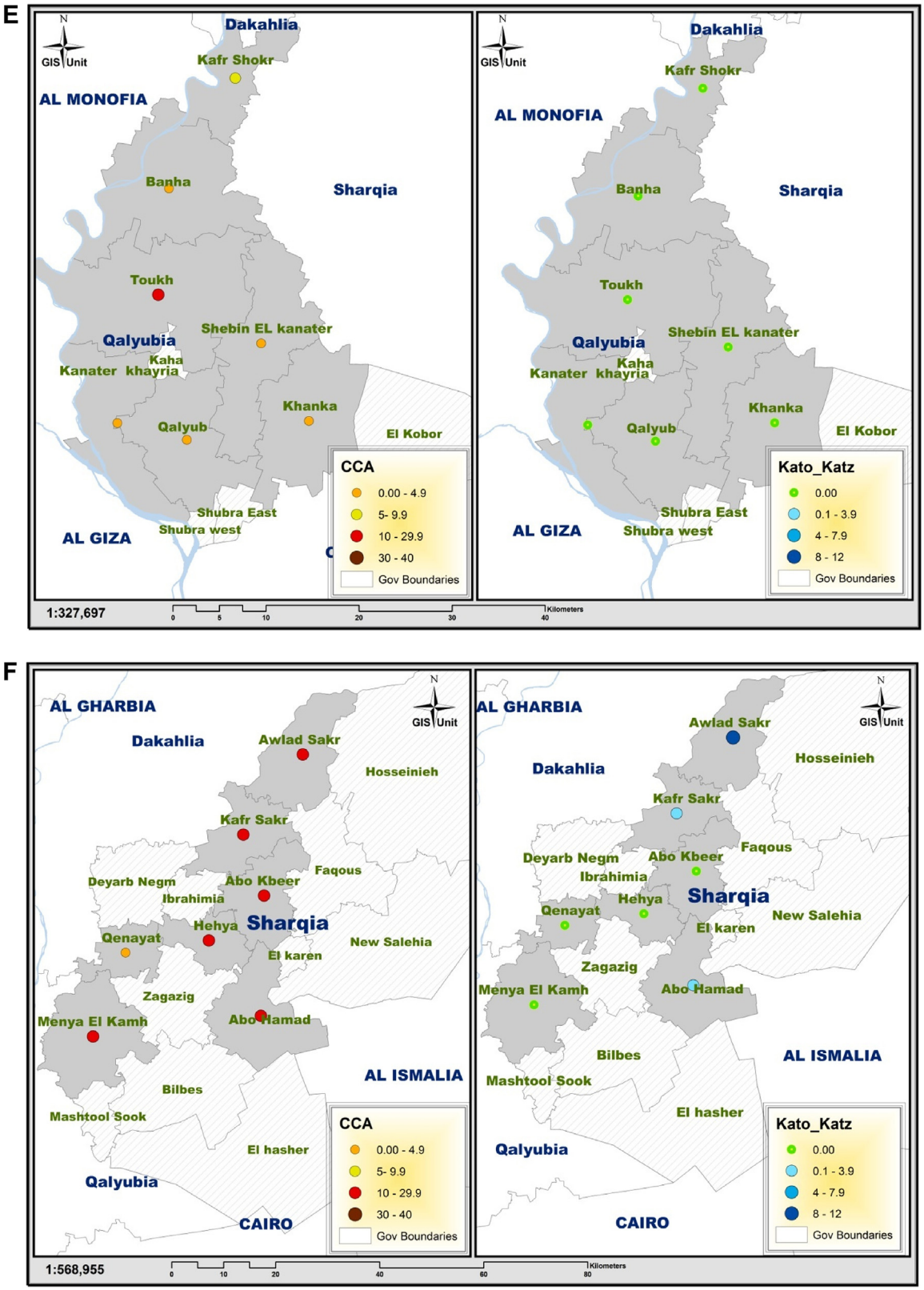

Fig 1. (*Continued*)

3. Results

3.1. Demographic characteristics of the study children

Overall 14,917 schoolchildren attending 151 schools were enrolled in the study. Of these children, 52% (n = 7751) were males and 48% (n = 7166) were females. These children attend primary and preparatory schools, their ages ranged between 6 and 12 (n = 10,811) and 13–15 years (n = 4106), respectively.

Table 1
Comparison of Kato-Katz and Urine-CCA prevalence rates in the five studied governorates.

Study area	Number of study participants tested	Kato-Katz prevalence rate (95% CI)	Urine-CCA prevalence rate (95% CI)	Chi-square	p-value
Sharqia Governorate	3000	3.5 (2.8%–4.2%)	17.6 (16.2%–19.0%)	316.0	P < 0.01
Kafr El Sheikh Gov.	5000	1.7 (1.3%–2.1%)	9.4 (8.6%–10.2%)	279.7	P < 0.01
Dakahlia Govnorate	2997	0.5 (0.2%–0.8%)	12.3 (11.1%–13.5%)	344.9	P < 0.01
Behira Governorate	974	0.4 (0.1%–0.9%)	9.7(7.9%–11.6%)	86.7	P < 0.01
Qalyubia Governorate	2946	0.03 (−0.03%–0.09%)	4.7 (3.9%–5.5%)	138.3	P < 0.01
Total	14,917	1.4 (1.2%–1.6%)	10.7 (10.2%–11.2%)	1134.9	p < 0.0001

Table 2
S *mansoni* prevalence rates in schoolchildren as determined be Kato-Katz and Urine-CCA.

Study area Governorate: District	Number of study participants tested	Kato-Katz prevalence rate (95% CI)	Urine-CCA prevalence rate (95% CI)
Sharqia Governorate:			
Awlad Sakr	1036	9.4 (7.6%–11.3%)	25.8 (23.1%–28.5%)
Kafr Sakr	450	1.3 (0.4%–2.8%)	16.0 (12.7%–19.7%)
Abo Kebeer	106	0.0	23.6 (15.8%–31.8%)
Hehya	300	0.0	16.0 (12.0%–20.6%)
Abo Hamad	312	0.6 (0.7% – 2.3%)	14.4 (10.7%–18.8%)
Menya El Kamh	495	0.0	12.3 (9.5%–15.5%)
Qenayat	301	0.0	3.7 (1.8%–6.4%)
Total	3000	3.5 (2.9%–4.2%)	17.6 (16.3%–19.0%)
Kafr El Sheikh Gov:			
Kafr El Sheikh	598	1.5 (0.7%–2.8%)	10.5 (8.2%–13.3%)
Desouk	919	1.2 (0.6%–2.1%)	9.8 (7.7%–11.5%)
Al Riad	400	0.0	10.8 (7.9%–14.2%)
Sidy Salem	800	0.9 (0.3%–1.8%)	7.6 (5.9%–9.7%)
Beyalah	482	1.7 (0.7%–3.2%)	6.6 (4.6%–9.2%)
Qeleen	400	0.0	5.0 (3.1%–7.6%)
Fowah	401	1.7 (0.7%–3.6%)	6.2 (4.1%–9.1%)
Balteem	200	3.0 (1.1%–6.4%)	15.5 (10.8%–21.3%)
Motobus	300	8.3 (5.5%–12.1%)	18.0 (13.8%–22.8%)
El Hamoul	500	2.6 (1.4%–4.4%)	10.0 (7.5%–12.9%)
Total	5000	1.7 (1.4%–2.1%)	9.4 (8.6%–10.2%)
Dakahlia Gov:			
Sherbeen	800	0.3 (0.03%–0.9%)	11.4 (9.3%–13.7%)
Bany Ebeed	300	0.0	8.7 (5.7%–12.4%)
El Manzalah	400	1.0 (0.02%–1.98%)	13.2 (10.0%–16.9%)
El Gamalia	84	3.6 (0.74%–10.0%)	32.1 (22.1%–43.2%)
Dekernes	100	0.0	26 (17.4%–34.6%)
Mit Selseel	100	4.0 (1.1%–9.9%)	30 (21.2%–39.9%)
Aga	613	0.0	4.1 (2.6%–5.9%)
Talkha	600	0.5 (0.1%–1.4%)	11.3 (8.9%–14.1%
Total	2997	0.5 (0.2%–0.8%)	12.3 (11.1%–13.5%)
Behira Governorate:			
Damanhour	575	0.0	8.3 (6.2%–10.9%)
Abo Homos	300	0.0	4.0 (2.1%–6.8%)
Al Mahmoudia	99	4.0 (1.1%–9.9%)	34.0 (24.8%–44.1%)
Total	974	0.4 (0.1%–0.9%)	9.7 (7.9%–11.6%)
Qalyubia Gov:			
Toukh	1196	0.08 (0.002%–0.4%)	10.5 (8.3%–11.7%)
Shebin Kanater	200	0.0	1.0 (0.1%–3.5%)
Qalyub	400	0.0	0.0
Kafr Shokr	200	0.0	5.5 (2.8%–9.6%)
Kanater Khayria	200	0.0	0.0
Khanka	200	0.0	0.0
Benha	550	0.0	0.0
Total	2946	0.03 (0.0001%–0.2%)	4.7 (3.9%–5.5%)

3.2. *Prevalence of* S. mansoni *based on the kato-Katz (KK) technique*

The overall prevalence as determined by a single KK thick-smear was 1.4% (95% CI: 1.2% – 1.6%). The estimated prevalence rates by governorate were 3.5%, 1.7%, 0.5%, 0.4% and 0.03% in Sharqia, Kafr El Sheikh, Dakahlia, Behira and Qalyubia governorate, respectively (Table 1). Within each governorate, there was marked variation in district prevalence rates (Table 2 and Fig. 1). Except two districts "hot-spots", one in Sharqia (Awlad Sakr, 9.4%) and the other in Kafr El Sheikh (Motobus, 8.3%), the *S. mansoni* prevalence by KK in all districts was ≤4% (Table 2 and Fig. 1). In Awlad Sakr district (Sharqia Governorate), where the highest prevalence was recorded, school prevalence rates ranged between 28% and zero percent (median = 7%). In Motobus district (Kafr El Sheikh Governorate), school prevalence rates ranged between 10% and 6% (median = 9%).

3.3. *Prevalence of* S. mansoni *based on the urine-CCA technique*

The overall prevalence by a single Urine-CCA test was 10.7% (95% CI: 10.2% – 11.2%), higher than that of the KK technique, p < 0.0001 (Table 1).The governorate prevalence rates were 17.6%, 9.4%, 12.3%, 9.7% and 4.7% in Sharqia, Kafr El Sheikh, Dakahlia, Behira and Qalyubia governorate, respectively. Table 2 summarizes the prevalence rates obtained in the different study areas, as assessed by the two diagnostics approaches. The 95% confidence intervals (95% CI) for prevalence are shown. In general, the Urine-CCA prevalence rates in the studied districts and governorates were significantly higher than those determined by Kato-Katz (p < 0.01).

Of 35 districts surveyed in the five governorates, *S. mansoni* infection was detected in 19 districts (54.3%) using KK, and in 31 districts (88.6%) by Urine-CCA ($\chi 2 = 9.94$; P = 0.0016), i.e. *S. mansoni* infection was detected by Urine-CCA, but not by KK in 12 districts (34.3%). *S. mansoni* infection was not detected by either of the two diagnostic methods in four districts (2123 schoolchildren attending 22 schools), all in Qalyubia governorate.

3.4. *Performance of the urine-CCA in different endemicity settings*

According to the Kato-Katz prevalence, studied schools were grouped in three endemicity settings; (a) moderate endemicity setting (KK prevalence of ≥10%); (b) low endemicity setting (KK prevalence of <10%); and (c) zero KK prevalence (Table 3). In the moderate endemicity setting (a) of 510 urine samples tested, 174 samples were Urine-CAA positives and judged 3+ (Fig. 2). The Urine-CCA prevalence (34.1%) was approximately doubles that of KK (17.3%). In the low endemicity setting (b), of 5133 urine samples tested, 606 samples were Urine-CCA positives and judged 1+ (Fig. 2). The Urine-CCA prevalence (11.8%) was approximately six times that of KK (2.1%). In the zero KK prevalence group, of 7151 urine samples tested, 718 samples were Urine-CAA positives and judged Trace (Fig. 2), and the prevalence was 10%. Overall, approximately 10% of the Urine-CCA positive samples were scored 3+, 40% 1+, and 50% trace. Note that the Urine-CCA tests scored Trace were mostly faint-very faint bands.

3.5. *Association between urine-CCA prevalence and, age and gender of schoolchildren*

The studied males (7751) were 1.2 times as likely to be infected (Urine-CCA infection rate 11.7% [95% CI: 11.0-12.4]) as females (7166; 9.7% [95% CI: 9.0-10.4]); $\chi 2 = 15.0$, p < 0.0001. In addition, the higher age groups (9->12 years; 10.7% [95% CI: 9.9% –11.4%]) and 12–15 years; 11.9% [95% CI: 10.9% –12.9%]) were 1.2 and 1.3

Table 3
S. mansoni infection rates in the different endemicity settings as categorized by Kato-Katz stool examination.

Endemicity settings as determined by Kato-Katz method	Number of schools	Number of study participants tested	Kato-Katz prevalence rate (95% CI)	Urine-CCA prevalence rate (95% CI)	Chi-square	p-value
Moderate endemicity (KK > 10%)	5	510	17.3 (14.0%–20.6%)	34.1 (30.0%–38.2%)	38.0	P < 0.01
Low endemicity (KK < 10%)	51	5133	2.1 (1.7%–2.5%)	11.8 (10.9%–12.7%)	164.6	P < 0.01
Areas with negative test (KK = 0)	73	7151	0.0	10.0 (9.3%–10.7%)	344.9	P < 0.01

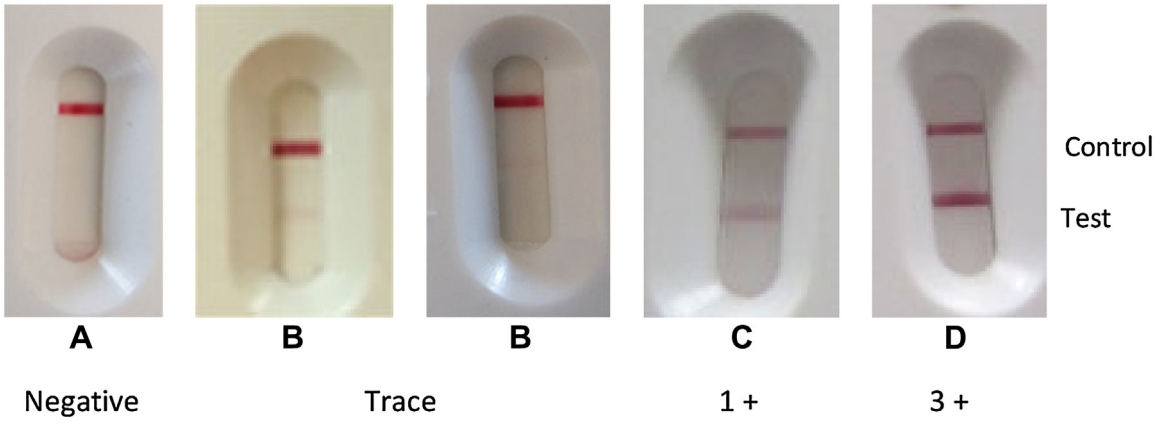

Fig. 2. Photograph of the different observed reactions of the Urine-CCA: (A) Negative, (B) Trace, (C) Positive (1+), and (D) strong positive (3+).

Table 4
S. mansoni infection rates as determined by Urine-CCA in the different schoolchildren age groups.

Governorate	Age Group	Total Number tested	Urine-CCA prevalence rate (95% CI)	Odds ratio	Chi-square	P-value
All	6–<9	4189	9.6 (8.8%–10.6%)	1		
	9–<12	6622	10.7 (9.9%–11.4%)	1.12	10.6	0.001
	12–<15	4106	11.9 (10.9%–12.9%)	1.26		
	Total	14,917	10.7 (10.2%–11.2%)			
Sharqia	6–<9	945	13.4 (11.3% –15.7%)	1		
	9–<12	1281	18.2 (16.1%–20.4%)	1.44	20.1	0.0001
	12–<15	774	21.5 (18.75 –24.6%)	1.77		
	Total	3000	17.6 (16.2% –19.0%)			

There is a trend for higher *S. mansoni* infection (as determined by Urine-CCA) among older age-group students 12–<15 compared to other age categories.

times as likely to be infected as the youngest age group (6- < 9 years; 9.6% [95% CI: 8.8% −10.6%]) (Table 4).

Age and gender infection trends were further studied in Sharqia governorate, with the highest prevalence rates. Children with age groups 9–12 years; 18.2% [95% CI: 16.1%–20.4%] and >12 years; 21.5% [95% CI: 18.75%–24.6%] were 1.4 and 1.8 times as likely to have Urine-CCA positive test as younger age group (6–<9 years; 13.4% [95% CI: 11.3%–15.7%]) (Table 4). Males were 1.6 times as likely to be infected (Urine-CCA positive) as females (Table 5).

4. Discussion

Currently, schistosomiasis control strategies implemented by MoHP rely on extensive screening exercises of the population living in rural areas in order to decide where mass treatment should be implemented. Screening of *S. mansoni* infection, through active case-detection conducted during surveys in schools and villages as well as through passive case-detection in communities at Rural Health Units, is based on single KK thick smear. The KK has limited sensitivity, especially in situations where light intensity infections are common, such as in areas where prevalence of infection is low as a result of mass treatment with praziquantel or other interventions. In low-prevalence settings, it is thus very difficult to achieve a conclusive confirmation on the infection status of screened individuals and consequently on the true levels of infection at the population level.

In compliance with WHO recommendations for areas of low endemicity (WHO, 2013), the MoHP in Egypt is planning for extensive MDA to eliminate *S. mansoni* in all areas of residual transmission. It was therefore necessary to update the distribution of the infection by a more sensitive diagnostic tool, in order to ensure that no endemic area would be excluded from the intervention. As such, assessment of the prevalence of *S. mansoni* infection was carried out in schoolchildren by the commercially available Urine-CCA cassette test. The Urine-CCA test, detects an adult worm antigen (CCA), which is secreted in infected host urine, and has been shown to be at least as sensitive as 3–9 KK thick smears (Utzinger et al., 2001; Colley et al., 2013). For comparative purposes, a single Kato-Katz, the most widely used technique for epidemiological field surveys, and the one adopted by the MoHP's NSCP, was also carried out.

The calculated prevalence rates, based on a single KK technique, are consistent with previous MoHP data (WHO, 2007; WHO, 2011) and clearly indicate that most of the endemic settings are of low endemicity (≤3%). Furthermore, the finding of two "hot spots" by KK (in Sharqia and Kafr El Sheikh Governorates) is in line with the current *S. mansoni* epidemiological picture in the Nile Delta (WHO, 2011). However, it is known that the routine (single) KK underestimates the "true" prevalence of infection, as shown by other surveys, which demonstrated that repeated examinations resulted in increasing the sensitivity of the method, and consequently in increasing estimates of the prevalence of *S. mansoni* (Coulibaly et al., 2011; Colley et al., 2013; Kittur et al., 2016; Siqueira et al., 2016).

Table 5
Comparison of *S. mansoni* prevalence in female and male schoolchildren as distributed by age groups.

Governorate	Age Group	Total Number tested	Male		Female		chi-square	Odds ratio	95% CI	
			Number tested	Urine-CCA positive%	Number tested	Urine-CCA positive%				
Sharqia	6-<9	945	507	14.6	438	12.1	1.3			0.2627
	9-<12	1281	614	21.8	667	15.0	9.9	1.58	1.2–2.1	0.0017
	12–15	774	459	25.5	315	15.9	10.2	1.81	1.2–2.7	0.0015
	Total	3000	1580	20.6	1420	14.3	4.4	1.6	1.2–1.8	0.0001

Males were 1.6 times as likely to be infected, determined by Urine-CCA, as females.

This was confirmed by our survey, which showed that the *S. mansoni* prevalence as determined by the Urine-CCA was significantly higher than that obtained by the KK, being double in the moderate endemicity level and six times in the low endemicity level. Other studies (Tchuem Tchuenteí et al., 2012; Colley et al., 2013) have confirmed the high sensitivity of the Urine-CCA method by showing a correlation between the Urine-CCA prevalence and the prevalence obtained by 9 KK. In support of the diagnostic specificity of Urine-CCA, a significant number of KK negative/CCA positive subjects were found to convert to Urine-CCA negative upon 1–2 cycles of praziquantel treatment (Mwinzi et al., 2015).

It is worth noting that this work was not intended to evaluate the performance of the Urine-CCA test. However, the finding that *S. mansoni* infection was not detected by either of the two diagnostic methods in four districts (2123 schoolchildren attending 22 schools), in Qalyubia governorate has implication for the test specificity. This finding indicates the high specificity of the test, in particular absence of "false positive results" in areas non-endemic for *S. mansoni* or where the transmission has been interrupted.

The current MoHP treatment strategy employs KK prevalence thresholds to decide where population groups (schoolchildren or entire population of a village or sub-village) should be treated. The prevalence determined by the Urine-CCA test, however, implies that there is a significant unaddressed reservoir of *S. mansoni* infection. As such, the current MoHP's approach might prove inadequate to achieve elimination, as it would leave untreated a significant proportion of infected individuals who would contribute to sporadic contamination of the environment with infective eggs and hence sustain transmission.

Consequently, MoHP authorities adopted a new treatment strategy by readjusting thresholds for mass treatment to maintain schistosomiasis control pressure in all transmission areas. MoHP's readjustment took into consideration both prevalence based on KK and the prevalence based on Urine-CCA. In districts that have zero KK prevalence, but where *S. mansoni* infections were detected by Urine-CCA, all children attending schools in rural areas will be treated with praziquantel, and efforts will be made to reach out-of-school children. In districts that have KK prevalence >0% and <3% (all have higher Urine-CCA prevalence rates), MDA will be implemented in the village (and satellite villages) where surveyed schools are located. In districts that have KK prevalence of ≥3% (all have higher Urine-CCA prevalence rates), MDA will be implemented in all rural areas of the district. Treatment will be implemented for two successive years, supplemented with focal molluscicide for snail control, and then the *S. mansoni* endemicity levels will be re-evaluated. MoHP is now planning to complete Urine-CCA remapping in all other endemic governorates with the aim of identifying all areas of residual transmission where the elimination strategy should be applied.

Data of Qalyubia governorate are of special interest. It lies immediately north of Cairo in the Nile Delta region, east of the Damietta branch where the Nile divides into two main distributaries. Because of its geographical location and direction of water stream in the Nile, it is unlikely that infected *Biomphalaria* snails will be swept from irrigation canals in other governorates into Qalyubia irrigation canals. Besides, *S. mansoni* infection was not detected in four out of seven studied districts (57.1%) by either of the two diagnostic methods. It is possible that by the two-year efforts *S. mansoni* will be eliminated from the other three districts. Since the late 1990s *S. haematobium* had virtually disappeared from Qalyubia governorate (Habib et al., 2000). Thus, Qalyubia will possibly be the first schistosomiasis non-endemic governorate in Egypt. However, other stringent procedures for verification of elimination of schistosomiasis will have to be implemented (WHO 2009).

The age and gender infection trends, as determined by Urine-CCA, are typical of what has been described in prior investigations in Egypt (El Khoby et al., 2000; Habib et al., 2000). Males are more prone to infection than females because they are more likely to have been more exposed to infectious canal water due to cultural aspect of conservative rural societies. In addition, higher age groups (9- < 12, 12–15 years) are at higher risk of acquiring infection than the younger (6–<9 years) age group, as they are more likely to be employed in agriculture, and to harbor cumulative infections.

The present study had two limitations. The first was the potential bias of purposive sampling, which was adopted to maximise detection of ongoing transmission. The second was the possible overestimation of prevalence based on Urine-CCA positives. Urine-CCA positive tests in districts that have KK zero prevalence were Trace (mostly faint-very faint band). Some of them were hardly distinguished by some observers. Such faint coloured bands have also been observed in other studies, and there appears to be a strong correlation between the *S. mansoni* infection intensities and the intensity of CCA test bands (Tchuem Tchuenteí et al., 2012; Mwinzi et al., 2015; Kittur et al., 2016).

In conclusion, we updated the *S. mansoni* distribution map in five Nile Delta governorates using a more sensitive method (Urine-CCA). This work has added information on the performance of the Urine-CCA test in settings characterized by very low or no (0%) prevalence by KK. Based on the findings of the current *S. mansoni* mapping exercise, authorities of the MoHP adopted a new elimination strategy by readjusting thresholds for mass treatment with praziquantel and targeting all transmission areas. MoHP is now planning to proceed to remapping in all other endemic governorates using Urine-CCA, with the aim of identifying all areas of transmission where the more intensive elimination strategy should be applied.

Conflict of interest statement

We declare that we have no conflict of interest.

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performance of the study, data analysis and interpretation, the preparation of the manuscript, or the decision to publish.

Authors' contributions

A.H. participated in planning the study and co-supervised the field and laboratory work. A.R. participated in planning the study, co-supervised the field and laboratory work and served as the data manager. K.A. undertook the data analysis. A.G. participated in planning the study and in writing the manuscript. R.A. participated in planning the study. R.R. participated in planning the study, co-supervised the field and laboratory work, participated in data analysis and co-wrote the manuscript.

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