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IgM and IgG Antibody Enzyme Linked Immunosorbent Assay Profile Detected Using Adult *Schistosoma guineensis* Extract In Sera from Cameroonian Infected by *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma guineensis*

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Abstract: Schistosomiasis is endemic in Cameroon with three Schistosoma species infecting human in many areas. Data from many countries endemic for *S.mansoni*, *S.haematobium* and *S.japonicum* reported an age-dependent antibody response profile using homologous adult schistosome antigen extract. Less is known about the antibody profile in schistosomiasis endemic areas in Cameroon. This study aimed to assess the IgM and IgG antibody profiles using adult *S.guineensis* extract in immune sera from Schistosoma egg positive Cameroonians from intestinal or urinary schistosomiasis endemic areas. Whole adult *S.guineensis* antigen was extracted from a Cameroonian *S.guineensis* strain maintained in laboratory. The antigen extract was a supernatant obtained after centrifugation of grinded freeze-thawed 45 days old worms in enzyme inhibitor solution. Sera were obtained from residents of schistosomiasis endemic areas with microscopically confirmed monospecific infection by *S.guineensis*, *S.mansoni* or *S.haematobium* and Schistosoma free control sera from non endemic area. IgM and IgG antibody titers detection was proceeded by indirect ELISA using HRP-conjugated secondary IgM or IgG antibody. Optical densities (OD) were recorded with a spectrophotometer plate reader at 495 nm and mean OD were statistically analyzed according to age using Student t-test and egg load using ANOVA test. A total of 189 sera were tested. Sera from *S.haematobium*, *S.mansoni* and *S.guineensis* infections represented 68, 73 and 46 respectively. Of *S.mansoni* infection sera, 31 were collected at Lagdo and 42 in *S.mansoni* monospecific focus. Age had no significant influence on mean IgM and IgG antibodies OD although mean IgM and IgG changed with age. Mean IgG antibody increased with age in the three schistosomiasis sera. According to age, mean IgM decreased in *S.guineensis* infections sera, decreased from young to participants aged 15 to 24 years then increased in older in schistosomiasis *mansoni* sera. In schistosomiasis *haematobium* sera, mean IgM increased from young to participants aged 15 to 24 years then decreased in older. Egg load influenced significantly mean IgM and IgG titers. Mean IgM OD decreased with egg load in each of the schistosomiasis whereas mean IgG decreased in intestinal schistosomiasis sera. Mean IgM and IgG antibody titers detected with adult *S.guineensis* extract using ELISA technique in either intestinal or urinary schistosomiasis endemic areas in Cameroon were not age dependent but egg load depended on egg load in urine and stool samples. Diagnostic performance of the adult *S.guineensis* extract in antibody detection has been tested.

Keywords: Antibody, Optical Densities, Sera, ELISA, S.Mansoni, S.Guineensis, S.Haematobium, Infections, Cameroon

Introduction. Schistosomiasis is a helminthiasis of public health concern caused in humans by one or more of the six species of trematodes of the genus *Schistosoma* namely *S.haematobium*, *S.mansoni*, *S.guineensis*, *S.intercalatum*, *S.japonicum* and *S.mekongi*. The 2016 World Health Organization (WHO) report estimated at more than 3.5 millions DALYs (Disease Adjusted Life Years) the burden of schistosomiasis in 2015 worldwide, with the highest burden of schistosomiasis found in the WHO African

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Region (WHO, 2017). Transmission occurs mainly in tropical areas of Asia, Africa and America where humans and other mammals are infected by skin penetration of forked-tail cercariae shed by specific snail intermediate host contained in freshwater. After penetration, each cercariae transforms into a schistosomulae which migrates to portal system where it matures, males and females mate, then females lay eggs in elective sites according to Schistosoma specie (Ross et al., 2002). Most eggs infiltrate many organs and other are voided in stool or urine where they can be detected using microscopy techniques. Eggs entrapped and immune-complexes deposits in organs are the main cause of the pathology reported in schistosomiasis which determine either an intestinal or urogenital schistosomiasis (Ross et al., 2002). Although most of the infected subjects are symptomless mostly in transmission settings, the pathology of schistosomiasis is complex and infected can suffer from acute to chronic disease. In moderate to heavy infections, schistosomiasis may result in chronic infection with complications which vary according to schistosomiasis form. In intestinal schistosomiasis, symptoms which are commonly reported are diarrhea and blood in the stool; liver and spleen enlargement, and portal hypertension may occur in advanced cases. Haematuria is the predominant symptom in urogenital schistosomiasis which in chronic infection can develop fibrosis of the bladder and ureter, hydronephrosis and bladder cancer. Female genital schistosomiasis can develop and sometimes pathology of the seminal vesicles and prostate develop in males.

The epidemiology of schistosomiasis in Cameroon is most complex than other countries since three Schistosoma species namely *S.haematobium, S.mansoni* and *S.guineensis* are all endemic with many coendemic foci reported in many areas (Ratard et al., 1990, 1992, Brooker et al., 2000; Tchuem Tchuente et al., 2012, 2013).

Each of the schistosome stage which evolves in the definitive host was reported to induce a complex cellular and antibody immune response which result in part in the schistosomiasis pathogenesis and later to establishment of either a partial resistance against challenge infection. Previous reports indicated that adult schistosome is made of a mosaic of antigens moieties including cross-reactive antigens, gender specific antigen and species specific antigens (Druilhe *et al*. 1981; Hayunga *et al*., 1981; Hayunga *et al*., 1982a,b; Norden and Strand, 1984). Using each of the schistosome stage extract in antibody detection studies, the antibody profile in animal model as well as in residents living in monospecific endemic areas for schistosomiasis *mansoni* and schistosomiasis *haematobium* showed a complex antibody profile dominated by IgM, IgG isotypes and IgE (Dunne *et al*., 1987; Khalife *et al*., 1989; Naus et al., 1998, 2003). Antibody response titers were showed to change with the age of the infection as detected in sera of experimentally *S.mansoni* infected mouse with cercariae extract (Hayunga et al., 1987a) and macaque monkey with adult extract (Wilson et al.,2008) in which IgM appeared early in the first week postinfection then decreased while IgG appeared two weeks postinfection, increased and remained high during the infection. In schistosomiasis *mansoni* as well as schistosomiasis *haematobium* endemic areas in Africa, young infected residents had high IgM titers and low IgG titers whereas adult had high IgG titers and low IgM titers (Butterworth et al., 1985, 1996; Wilkins *et al*.,1987). In an immunoepidemiological study carried in Cameroon by antibody detection using ELISA technique, soluble *S.haematobium* egg extract detected in *S.haematobium* infected children higher IgM, IgE, IgG1, IgG2, IgG3 and IgG4 than soluble adult *S.haematobium* extract, the later detected high IgM and IgG3 and low IgE, IgG1, IgG2 et IgG4 antibody titers (Naus et al., 1998). A similar study carry out on IgG istotypes antibody detection in a schistosomiasis *mansoni* endemic area in Uganda reported predominant IgG4 titers in younger infected subjects with a also a susceptibility to reinfection whereas in older infected residents adult had a partial resistance to reinfection and predominant IgG1, IgG2 and IgG3 antibody titers (Naus et al., 2003). The role of each of the antibodies against reinfection was ambiguous some being were positively associated with susceptibility and others having positive association with resistance to Schistosoma reinfection. Due to their association with resistance to infection or reinfection, or their in vitro ability to kill schistosomulae, antibodies raised against schistosomiasis were classified as immunoprotective including IgG2, IgG3, IgE, IgA and IgG1 and non protective antibodies including IgM and IgG4 (Dunne *et al*., 1987; Khalife *et al*., 1989).

The importance of studying the antibody profile against a known antigen extract is incontrovertible as its allow identification of a specific schistosomiasis immunodiagnostic marker and also identification of immunological marker of resistance as well as susceptibility to infection or reinfection. Concerning the immunodiagnostic importance, almost all commercial schistosomiasis immunodiagnostic tests available are antibody detection tests made of whole extract of adult *S.mansoni*. Demonstrating the antibody responses pattern towards other Schistosoma species extract allow identification of potentially new diagnostic aid for schistosomiasis.

Almost all antibody detection studies have been undertaken monospecific foci of *S.mansoni*, *S.haematobium* and *S.japonicum* infections using homologous antigen extract. Few if any study has tested the antibody detection of an *S.guineensis* antigen extract, nor in *S.guineensis* infected. In Cameroon, where three Schistosoma species are coendemic, study of the antibody profile seemed also complex between monospecific and coendemic foci. Also, choosing a specific Schistosoma extract for specific schistosomiasis immunodiagnostic seems also complex in Cameroon setting. This study aimed to describe the pattern of IgM and IgG antibody profile by indirect ELISA technique using an adult *S.guineensis* extract with respect to age and egg load in Cameroonians living in schistosomiasis endemic areas harboring monospecific infection by *S.mansoni*, *S.haematobium* and *S.guineensis* extract, and also evaluate the impact of Schistosoma species coendemicity on the antibodies titers.

Material and methods.

Study area, patients and sera

Infection sera were extracted from fresh blood collected via venipuncture from residents in known schistosomiasis endemic areas with microscopically confirmed monospecific *S.mansoni* or *S.guineensis* or *S.haematobium* infections. Patients with *S.mansoni* infections were recruited at Nkolbisson (Centre Region, Cameroon) and Gounougou (Lagdo dam area, North region of Cameroon). Patients with *S.guineensis* infections were recruited among dwellers of Pongo quarter at Edea town (Cameroon) while patients with *S.haematobium* infection were recruited at Gounougou and Ouro-Doukoudje (Lagdo dam area, North region of Cameroon). Nkolbisson is an area always described as a monospecific *S.mansoni* focus whereas Gounougou and Ouro-Doukoudje are mixed foci for *S.mansoni* and *S.haematobium* (Ratard et al., 1990, 1992). Edea has always been described as a *schistosomiasis guineensis* monospecific focus (Ripert et al., 1981, 1987; Ratard et al., 1990). Control sera provided by the Immunology laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé1 (Cameroon) and were obtained from subjects who had never visited any schistosomiasis endemic area. Both infection and control sera were stored in 100µl aliquots at -20°C until use.

Ethics

After information of residents of the selected villages about the study objective and protocol, each volunteer village dweller had to sign the study inform or sign the study inform consent for his child (if the child was less the than 15 years old) before his inclusion in the study. Each eligible participant had to provide a stool sample and a urine sample. A 3 ml blood was then punctured from a hand vein of participants who provided the two samples for anti-Schistosoma antibody detection tests.

Stool and urine samples were analyzed the same day using Kato-Katz and urine filtration methods respectively. A praziquantel treatment was immediately given to all Schistosoma positive participants. Serum was extracted from each blood sample, aliquoted and stored at -20°C until use.

Origin of adult *S.guineensis* strain

S.guineensis eggs were originally isolated from fresh stool samples collected from dwellers Edéa town (Cameroon) with microscopically confirmed *S.guineensis* infection. Then *S.guineensis* life cycle has been routinely maintained in our laboratory through successive passage in the specific snail intermediate host *Bulinus forskali* and albinos mice as definitive host. *S.guineensis* eggs isolated by filtering fresh stool samples diluted in 50 volumes of 1.7% NaCl. The last sediment containing *S.guineensis* eggs was immediately diluted with filtered pond water or distilled water for egg hatching. Resulting miracidia were used to infect young inbred *B.forskalii* snail. Cercariae shed two to three weeks later by infected snails were used to infect one month old albinos mice by tail immersion (Erickson, 1974). Adult *S.guineensis* were recovered from infected mice 45 days post-infection by portal hepatic perfusion method with a saline perfusion solution (0.75% natrium citrate and 0.85% natrium chloride) as described by Duvall and DeWitt (1967).

Freshly recovered adult *S.guineensis* were washed 3 times with sterile perfusion solution and 3 times with phosphate-buffered saline (PBS) pH 7.2 before storage at -20°C in groups of 20 worms in a minimum volume of PBS pH 7.2 until use.

Preparation of adult *S.guineensis* antigen extract and protein content determination

A group of twenty frozen adult *S.guineensis* were resuspended in 1 ml of enzyme inhibitor (1mM phenylmethylsulfonyl fluoride) and thawed at room temperature and grinded on an ice bath in a Ten Broeck tissue grinder with a Teflon pestle. The homogenate was centrifuged at 5000 rt/min for 10 minutes and the supernatant was considered as adult worm antigen (AWA) extract and used for antibody detection in sera. The protein concentration of AWA was determined using the Bradford colorimetric method (Bradford, 1976).

IgM and IgG antibody Enzyme linked immunosorbent assay titration in control and schistosomiasis infection sera

Antibody titration was processed using indirect Enzyme linked immunosorbent assay technique as described earlier (Hillyer et Gomez de Rios,1979). ELISA procedure for titration of IgM and IgG antibodies with adult S.guineensis extract consisted in coating round bottom wells of a polystyrene microtitration plate (Costar) in duplicate with 100 µl of a 5µg/ml of AWA in carbonate buffer pH 9.6. The coated plate was incubated overnight at +4°C, then each plate was washed three times with 0.05% Tween 20 in PBS pH 7.2 (0.05% PBS-Tween 20). Plate was then dried on a blotting paper and 100 µl of 1% bovine serum albumin in PBS pH 7.2 (1% BSA) was added to each test well and the plate was incubated again for 1h at room temperature. The excess 1% BSA was discarded. Without washing, 100 µl of each serum diluted 1/500 of schistosomiasis *haematobium*, schistosomiasis *mansoni*, schistosomiasis *guineensis* or control was added to two consecutive wells and incubated for 2h at room temperature. Unfixed serum antibodies were discarded and the plate was washed five times at 5 min interval with 0.05% PBS-Tween 20. Each plate was incubated for 1h at room temperature with 100 µl per well of HRP-conjugated goat anti-human IgG or goat anti-human IgM (Sigma, St. Louis, Mo.) at a dilution of 1:4,000 in 1% BSA. Plates were washed again three times with 0.05% PBS-Tween 20 at 5 min interval then washed again 2 times with PBS pH 7.2. 100µl of ortho-phenylene diamine (OPD) plus H2O2 was added to each and the reaction was allowed to proceed in the dark for 30 min at 37°C. The reaction was stopped by adding to each well 50µl 4M H_2SO_4 and the absorbance was read at 492 nm with a microplate spectrophotometer reader (Biotrak II Reader). The final IgM or IgG optical density for each serum was calculated as the mean of the two optical densities recorded in the duplicated wells.

Data were recorded in SPSS software and statistically analyzed using the Student-t test for comparison of mean optical densities according to egg loads, and the analysis of variance (ANOVA) test for comparison of mean optical densities between age groups. Difference was considered significant at 5% for both statistical tests.

RESULTS.

A total of 189 sera were used in the study including 2 control sera, 68 *S.haematobium* infection sera, 73 *S.mansoni* infection sera and 46 *S.guineensis* infection sera. Of the *S.mansoni* infection sera, 31 sera were collected in Lagdo area and 42 were collected in the *S.mansoni* monospecific focus at Nkolbisson. Schistosomiasis patients were classified in three age groups: 5 years to 14 years, 15 years to 24 years, and over 24 years.

As indicated in table I, participants aged between 5 and 14 years were predominant among *S.haematobium* and *S.guineensis* groups whereas there was no significant difference in age groups representation among *S.mansoni* infected subjects. According to Schistosoma sp egg loads in feces or urine, subjects included in this study harbored either light infection or moderate infection. Among either intestinal schistosomiasis carriers or urinary schistosomiasis subjects, low intensity of infection carriers were the most represented groups.

Table I. Distribution of Schistosoma infected subjects according to age and egg loads in excreta.

Demographic	**Ranges**	**Schistosomiasis type**		
		S.mansoni	***S.haematobium***	***S.guineensis***
Age groups (years)	5-14	25 (34.2%)	31 (45.6%)	23 (50%)
	15-24	25 (34.2%)	19 (27.9%)	10 (21.7%)
	≥25	23 (31.6%	18 (26.5%)	13 (28.3%)
	Total	**73 (100%)**	**68 (100%)**	**46 (100%)**
Egg loads in feces (egg/g of feces)	1-99	60 (82.2%)	NA	31 (67.4%)
	≥ 100	13 (17.8%)	NA	15 (32.6%)
Egg loads in urine (egg/10 ml of urine)	1-49	NA	42 (61.8%)	NA
	≥ 50	NA	26 (38.2%)	NA

NA: non applicable.

IgM and IgG optical densities recorded in each schistosomiasis serum was higher than optical densities in each control serum.

Influence of age of participants on mean IgM and IgG antibodies OD in intestinal and urinary schistosomiasis sera

As indicated in table II, age had no significant influence on mean IgM and IgG antibodies optical densities. However, mean IgM and IgG antibody OD showed a fluctuation with age of participants in any of the three schistosomiasis sera group.

Table II. Influence of patient age on the pattern of IgM and IgG mean optical densities in *S.mansoni*, *S.haematobium* and *S.guineensis* infection sera.

Age groups (years)	**Schistosomiasis infection sera**								
	Schistosomiasis *mansoni*			**Schistosomiasis *haematobium***			**Schistosomiasis *guineensis***		
	N	**IgM**	**IgG**	N	**IgM**	**IgG**	N	**IgM**	**IgG**
5-14	25	0,226±0,09 [0,09-0,41]	0,174±0,09 [0,01-0,33]	31	0,173±0,049 [0,09-0,29]	0,168±0,06 [0,06-0,33]	23	0,265±0,07 [0,13-0,40]	0,251±0,10 [0,08-0,54]
15-24	25	0,164±0,07 [0,05-0,32]	0,22±0,067 [0,09-0,37]	19	0,195±0,082 [0,09-0,41]	0,174±0,07 [0,09-0,36]	10	0,232±0,07 [0,09-0,34]	0,264±0,12 [0,07-0,43]
≥ 25	23	0,177±0,08 [0,05-0,40]	0,236±0,08 [0,03-0,41]	18	0,167±0,071 [0,06-0,31]	0,174±0,055 [0,08-0,25]	13	0,207±0,08 [0,08-0,36]	0,29±0,11 [0,15-0,51]
ANOVA		3,736	4,07		0,933	0,213		2,554	0,532
df	70x2			*65x2*			43x2		

N: sample size.

In schistosomiasis *mansoni* infection sera, mean IgM optical densities showed a significant decrease with age between 5 to 14 years old subjects and 15 to 24 years old subjects then increased in older subjects (p <5%) whereas mean IgG optical densities showed a significant regular increase with age.
In Schistosomiasis *haematobium* sera, mean IgM antibody optical densities showed an increase but not significant with age then decrease in older subjects while mean IgG antibody optical densities showed a constant but not significant increase with age (p>5%).

In *S.guineensis* infection sera, age of patients did not significantly influence both mean IgM or IgG optical densities. However, a non significant decrease was recorded with age in mean IgM optical densities whereas mean IgG optical densities showed a regular but also not significant increase with age (p>5%).

Influence of parasitic load on mean IgM and IgG optical densities in intestinal and urinary schistosomiasis sera
According to table III, egg load significantly influenced both mean IgM ang IgG titers in schistosomiasis sera. Mean IgM titers significantly increased with egg loads in feces in schistosomiasis *mansoni* and schistosomiasis *guineensis* but showed a significant decreased in schistosomiasis *haematobium*. Mean IgG titers followed similar trends in both intestinal and urinary schistosomiasis. Mean IgG titers significantly increased with egg load in feces for schistosomiasis *mansoni* (t test: 16.77; df: 71), schistosomiasis *guineensis* (t test= 13.44; df: 44) and schistosomiasis *haematobium* (t test= 20.09; df:66).

IgM and IgG pattern in schistosomiasis *mansoni* sera from monospecific area and mixed *S.mansoni/S.haematobium* mixed area
IgM DO had similar ranges in the two foci while IgG DO range was wider in the mixed *S.mansoni*/*S.haematobium* mixed focus than in the monospecific *S.mansoni* focus. However, mean IgG DO was higher in sera from the *S.mansoni* monospecific focus than those from *S.mansoni*/*S.haematobium* mixed focus.

Participant age and *S.mansoni* egg had no significant influence on mean IgM and mean IgG titers in sera of *S.mansoni* infected participants from Lagdo and the monospecific *S.mansoni* focus in Nkolbisson. However, a decrease in mean IgG titer was noticed with *S.mansoni* egg load in the two areas whereas mean IgM titer increased with parasitic load in the *S.mansoni/S.haematobium* focus. In the *S.mansoni* monospecific endemic area (Nkolbisson), mean IgM titer showed decreased with egg load.

Discussion
The aim of the study was to assess the antibody response pattern using ELISA technique with HRP secondary antibody detection in sera from intestinal schistosomiasis as well as urinary schistosomiasis in Cameroon with special reference to ELISA IgM and IgG.

The Enzyme linked immunosorbent assay (ELISA) is the most widely recommended in titration of antibody compounds. HRP conjugated secondary antibody is also of must used reagents in ELISA detection of primary antibody in sera.

Results gathered indicated higher antibody titers in immune schistosomiasis infections sera than in

control sera indicating presence of specific antibodies directed towards specific antigens in adult *S.guineensis* extract. Occurrence of both IgM and IgG specific titers in schistosomiasis sera showed a wide range in other of the three schistosomiasis infections sera tested. Such tendency was reported in field studies in schistosomiasis *mansoni* as well schistosomiasis *haematobium* endemic areas using homologous antigen extract in indirect ELISA technique (Naus et al, 1998, 2003; Butterworth et al.,1985; Wilkins et al.,1987; Woolhouse *et al*., 1991; Hagan et al.,1991; Dunne et al., 1992; Naus et al., 2003 ;Elfaki et al., 2016). In this study, mean IgG titers decreased significantly with age in all the three schistosomiasis tested whereas IgM titers showed different trend in intestinal and urinary schistosomiasis. Data gathered in this indicated significant increase in mean IgM titers in both schistosomiasis *mansoni* and schistosomiasis *guineensis* sera. Such increase in IgM titers did not corroborate reports from many studies carried either in schistosomiasis *mansoni* (Butterworth et al., 1985,1996) or schistosomiasis *haematobium* endemic (Wilkins et al., 1987 ; Naus etal., 1998, 2003) areas which always reported higher IgM titers in younger participants sera. We were expecting similar tendency at least in homologous infection (schistosomiasis *guineensis*). In those studies in schistosomiasis endemic areas, high IgM titers as well as high IgG4 titers were significantly associated with susceptibility to infection and reinfection. However, in schistosomiasis *haematobium* sera, mean IgM tiers showed a decrease with age corroborating previous studies in endemic areas which used extracts from adult or egg of *S.haematobium* (Wilkins et al., 1987 ; Naus et al., 1998, 2003).

Data gathered in IgG titration indicated a decrease with age in the both homologous and heterologous Schistosoma infections which was a different trend from data gathered in immunoepidemiology studies in either schistosomiasis *mansoni* (Butterworth et al., 1985,1996) or *S.haematobium* (Wilkins et al., 1987 ; Naus et al., 1998, 2003). In these previous, studies, some IgG isotypes namely IgG1, IgG2 and IgG3 showed significant increase with age whereas IgG4 decreased in older participants. The decrease recorded in IgG titers in this study may therefore be due to a predominance of susceptibility-associated IgG isotype in younger participants. Therefore, IgG titers detected by antigens in the adult *S.guineensis* extract may therefore be predominantly susceptible-associated isotypes.

Studying immunoepidemiology of schistosomiasis is interesting in many ways. One interesting aspect of this immunoepidemiology study rely on the fact, further identification of antibodies associated to any susceptibility or resistance to reinfection could be a useful tool for post-therapeutic follow-up. Such a relationship has been reported in reinfection studies after praziquantel treatment in monospecific schistosomiasis *haematobium* focus in Cameroon (Naus et al., 1998) and other monospecific schistosomiasis in other African countries Butterworth et al.,1985; Wilkins et al.,1987; Woolhouse *et al*., 1991; Hagan et al.,1991; Dunne et al., 1992; Naus et al., 2003 ;Elfaki et al., 2016) where higher IgM and IgG4 positively correlated with higher susceptibility to reinfection whereas high IgG1, IgG2, IgG3 and IgE were positively associated with resistance to reinfection. Another interesting aspect was to further evaluate immunodiagnostic potential of adult *S.guineensis* extract ant also purified specific antigens. Assessing diagnostic performance of adult *S.guineensis* extract as well as other whole or purified extracts from Schistosoma species found in Cameroon will enable production of local and cheaper highly specific immunodiagnostic reagent which are urgently needed as Cameroon as well as other African schistosomiasis endemic countries are looking forward to move from schistosomiasis control to elimination. In this respect, the importance of immunodiagnosis has been reaffirmed since decades but the most limitation relies on the low specificity of commercially available antibody or antigen detection tests which need improvement (Mott and Dixon,1982; Mott et al., 1987; Grenfell et al., 2012).

Identifying antibody profile with respect to age and parasitic load could help improve interpretation of immunodiagnostic results. In this respect, an approach to distinguish specify Schistosoma infection with some ELISA-based antibody detection immunodiagnostic tests using soluble Schistosoma antigen extract was proposed in an immunodiagnostic study of *S.mansoni* and *S.haematobium* infections which reported that optical densities ratios recorded with CEF6 over *S.margrebowiei* egg extract indicated *S.mansoni* if the ratio<1 and a *S.haematobium* infection if the ratio >1 (Turner et al.,2004).

Developing immunodiagnostic tests from local schistosome parasites strains through routine maintenance of the parasite life cycle in laboratory will be cheaper than commercially available immunodiagnostic tests since almost all schistosomiasis countries are located in low and middle income countries.

Conflict of interests

The authors of this manuscript declare that they have no conflict of interest for this study.

Author's contributions

TK designed the study, collected field samples and laboratory data, analyzed data and wrote the manuscript. GL, AT, FN designed the study, analyzed data and red the manuscript. ASE designed the study, followed-up the study and read the manuscript.

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References

1. Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annal Biochem*, 72: 248-254.
2. Butterworth AE, Capron M, Cordingley JS, Dalton PR, Dunne DW et al. (1985). Immunity after treatment in human schistosomiasis *mansoni*. II : Identification of resistant individuals, and analysis of their immune responses. *Trans Roy SocTrop Med Hyg,79: 393-408.*
3. Butterworth AE, Dunne DW, Fulford AJC, Ouma JH, Sturrock RF (1996). Immunity and morbidity in *Schistosoma mansoni* infection: quantitative aspects. *Am J Trop Med Hyg, 55(5): 109-115*.
4. Druilhe P, Richard-Lenoble D, Gentilini M (1979). Parasite immunology. Encyl Med Chir, Paris. Infectious Diseases, 8078A10,5. [French].
5. Dunne DW, Butterworth AE, Fulford AJ, Kariuki HC, Langley JG et al (1992). Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol. 1992; 22(6):1483–94*.
6. ElFaki TEM, Arndts K, Wiszniewsky A, Ritter M, Goreish IA et al. (2016). Multivariable regression analysis in *Schistosoma mansoni*-infected individuals in the Sudan reveals unique immunoepidemiological profiles in uninfected, egg+ and non egg+ infected individuals. *Negl Trop Dis* 10(5): e0004629. Doi:10.1371/journal.pntd.0004629 May 6, 2016.
7. Erickson DG (1974). An efficient technique for exposure of rodents to *Schistosoma mansoni* or *Schistosoma haematobium*. *J Parasitol, 60: 553-554*.
8. Grenfell RFQ, Silva-Moraes V, Taboada D, deMattos ACA, de Castro AKS, Coelho PMZ (2012). Immunodiagnostic methods: what is their role in areas of low endemicity? The ScientificWorld Journal 2012, 5 pages. Article ID 593947. DOI:10.1100/2012/593947
9. Hagan P, Blumenthal UJ, Dunne D, Simpson AJG, Wilkins HA (1991). Human IgE, IgG4, and resistance to reinfection with *Schistosoma haematobium. Nature, 349:243-5*.
10. Hayunga EG, Sumner MP, Stek M Jr (1982a). Isolation of a Concanavalin A-binding glycoprotein from adult *Schistosoma japonicum*. *J Parasitol*, 68(5) : 960-961.
11. Hayunga EG, Sumner MP, Stek M Jr, Stirewalt MA (1982b). Characterization of surface glycoproteins on *Schistosoma mansoni* by lectin affinity chromatography and metabolic labeling using hexose precursors. *Mol Biochem Parasitol*, Supplement : 654.
12. Hayunga EG, Mollegard J, Duncan JF, Sumner MP, Stek M.Jr, Hunter KW Jr (1987). Early diagnosis of S. *mansoni* in mice using assays directed against cercarial antigens isolated by hydrophobic chromatography. *J Parasitol*, 73(2): 351-362.
13. Hayunga EG, Vannier WE, Chestnut RY(1981). Partial characterization of radiolabeled antigens from. adult *Schistosoma haematobium*. *J Parasitol*, 67(4) : 589-591.
14. Hillyer and Gomez de Rios (1979). Enzyme-Linked Immunosorbent Assay (ELISA) for the immunodiagnosis of schistosomiasis. *Am J Trop Med Hyg*, 28 : 237-241.
15. Mott KE, Dixon H (1982). Collaborative study on antigens for immunodiagnosis of schistosomiasis. *Bull World Health Organization*, 60(5): 729-753.
16. Mott KE, Dixon H, Carter CE, Garcia E, Ishii A et al. (1987). Collaborative study on antigens for immunodiagnosis of *Schistosoma japonicum*. *Bull World Health Organization*, 65(2) : 233-244.
17. Naus CWA, Van Dam GJ, Kremsner PG, Krijger FW, Deelder AM (1998). Human IgE, IgG subclass, and IgM responses to worm and egg antigens in Schistosomiasis *haematobium*: a 12-month study of reinfection in Cameroonian children. *Clinical Infectious Diseases 26:1142–7*. Downloaded from http://cid.oxfordjournals.org/ by guest on September 9, 2015
18. Naus CWA, Booth M, Jones FM, Kemijumbi J, Vennervald BJ et al. (2003). The relationship between age, sex, egg-count and specific antibody responses against *Schistosoma mansoni* antigens in a Ugandan fishing community. *Trop Med Int Health, 8(6) : 561–568*.
19. Norden AP, Strand M (1984). *Schistosoma mansoni S.haematobium* and *S. japonicum*. Identification of genus, species and gender-specific antigenic worm glycoproteins. *Exp Parasitol*, 57: 110-123.
20. Ratard RC, Kouemeni LE, Ekani Bessala MM, Damkou CN, Greer GJ, Spilsbury J, Cline BL (1990). Human schistosomiasis in Cameroon. I ; Distribution of schistosomiasis. *Am J Trop Med Hyg*, 42 : 561-572.
21. Ratard RC, Kouemeni LE, Ekani Bessala MM, Damkou CN (1992). Estimation of the number of cases of schistosomiasis in a country : the example of Cameroon. *Trans Roy Soc Trop Med Hyg*, 86 : 274-276.
22. Ripert C, Carrie J, Pagbe JJ, Ravinet L, Deniau M, Same-Ekobo A (1981). Epidemiological study of Eseka and Edea schistosomiasis *intercalatum* foci (Cameroon). Effect of praziquantel treatment. *Azrmeittel Forschung,31 : 584-589.*
23. Ripert C (1987). Epidemiology of *S.intercalatum* schistosomiasis in Central Africa. Effect praziquantel treatment. Ixth International Congress of Infectious and parasitic Diseases. *Med Chir Dig, 16(8) : 597-599.*
24. Ross AGP, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, Mcmanus (2002). Schistosomiasis: Current Concepts. *N Engl J Med*, 346(16) : 1212-1220. www.nejm.org
25. Tchuem Tchuente LA, Ngassam RIK, Sumo l, Ngassam P, Dongmo Noumedem C et al. (2012). Mapping of schistosomiasis and soil-transmitted helminthiasis in the regions of Centre, East and West Cameroon. *PLoS Negl Trop Dis* 6(3): e1553. Doi:10.1371/journal.pntd.0001553.
26. Tchuem Tchuente LA, Dongmo Noumedem C, Ngassam P, Kenfack CM, Gipwe NF et al. (2013). Mapping of schistosomiasis and soil-transmitted helminthiasis in the regions of Littoral, North-West, South and South-West Cameroon and recommendations for treatment. BMC Infectious Diseases 2013 13:602. *BMC Infectious Diseases*, 13:602. http://www.biomedcentral.com/1471-2334/13/602.
27. Turner P, Lalloo K, Bligh J, Armstrong M, Whitty CJM et al. (2004). Serological speciation of human schistosome infections by ELISA with a panel of three antigens. *J Clin Pathol*, 57: 1193-1196. Doi: 10.1136/jcp.2003.014779. Downloaded from http://jcp.bmj.com/ on December 8, 2016.

28. Wilkins HA, Blumenthal UJ, Hagan P, Hayes RJ, Tulloch S (1987). Resistance to reinfection after treatment of urinary schistosomiasis. *Trans Roy Soc Trop Med Hyg, 81: 29-35.*

29. Wilson RA, Langermans JAM, van Dam GJ, Vervenne RA, Hall SL, Borges WC, Dilon GP, Thomas AW, Coulson PS (2008). Elimination of *Schistosoma mansoni* adult worms by Rhesus Macaques: basis for therapeutic vaccine? *PLoS Negl Trop Dis* 2(9): e290. Doi:10.1311/journal.pntd.0000290. www.plosntds.org

30. Wilkins HA, Blumenthal UJ, Hagan P, Hayes RJ, Tulloch S (1987). Resistance to reinfection after treatment of urinary schistosomiasis. *Trans Roy Soc Trop Med Hyg,* 81 : 29-35.

31. World Health Organization (2017). Schistosomiasis and soil-transmitted helminthiasis: number of people treated in 2016. *Weekly Epidemiological Record*, 8 December 2017, 92th Year, 49(92): 749–760. http://www.who.int/wer.

Table III. Influence of infection intensities (egg load) on the pattern of IgM and IgG mean optical densities in *S.mansoni*, *S.haematobium* and *S.guineensis* infection sera.

	Intestinal schistosomiasis infection sera						Urinary schistosomiasis infection sera			
	Schistosomiasis *mansoni*			Schistosomiasis *guineensis*			Egg load range			
	N	IgM	IgG	N	IgM	IgG	(egg/10ml urines)	N	IgM	IgG
Overall mean OD	73	0,189±0,08 [0,05-0,41]	0,209±0,08 [0,01-0,41]	46	0,241±0,077 [0,08-0,40]	0,265±0,108 [0,07-0,54]		68	0,177±0,065 [0,06-0,36]	0,171±0,06 [0,06-0,36]
1-99	60	0,188±0,083 [0,05-0,40]	0,216±0,08 [0,01-0,41]	31	0,234±0,072 [0,08-0,40]	0,281±0,099 [0,15-0,54]	1-49	42	0,182±0,073 [0,06-0,41]	0,179±0,067 [0,06-0,36]
≥ 100	13	0,202±0,107 [0,09-0,41]	0,180±0,10 [0,03-0,39]	15	0,255±0,088 [0,11-0,40]	0,231±0,12 [0,07-0,41]	≥50	26	0,171±0,052 [0,09-0,31]	0,158±0,051 [0,08-0,25]
Student t test	df : 71	5,537	16,775	df :44	10,916	13,446		df 66	10,129	20,0965

Egg load were calculated as per 10 ml of urine for schistosomiasis *haematobium*, and as per g of feces for intestinal schistosomiasis.. N=sample size.

Table IV. Influence of *S.mansoni* egg load and participant age groups on mean IgM and IgG titers in schistosomiasis *mansoni* sera according to area.

Area		Overall mean OD	*S.mansoni* egg loads (egg/g of faeces)			Infected subjects age groups			ANOVA test
			1-99	≥100	t test	5-14	15-24	≥25	
Lagdo	**Sample size**	31	24	7		12	9	9	
	Mean IgM	0,204±0,09 [0,05-0,41]	0.199±0.09 [0.05-0.38]	0.235±0.12 [0.01-0.41]	0.828 df :30	0.242±0.1 [0.11-0.41]	0.185±0.09 [0.08-0.32]	0.167±0.08 [0.05-0.28]	F=1.514 df :28x2
	Mean IgG	0,192±0,09 [0,01-0,41]	0.196±0.09 [0.01-0.41]	0.186±0.13 [0.03-0.39]	2.015 df :30	0.141±0.08 [0.01-0.28]	0.207±0.07 [0.09-0.32]	0.25±0.115 [0.03-0.41]	F=1.32 df:28x2
	Sample size	42	36	6		12	16	14	
Nkolbisson	**Mean IgM**	0,179±0,078 [0,05-0,4]	0.18±0.077 [0.05-0.4]	0.17±0.09 [0.09-0.33]	0.279 df :41	0.208±0.08 [0.09-0.33]	0.152±0.06 [0.05-0.28]	0.184±0.08 [0.07-0.4]	F=1.8285 df :39x2
	Mean IgG	0,221±0,07 [0,06-0,37]	0.229±0.07 [0.06-0.37]	0.175±0.07 [0.08-0.25]	1.6899 df :41	0.209±0.09 [0.08-0.33]	0.228±0.065 [0.12-0.37]	0.226±0.06 [0.06-0.31]	F=11.3154 df :39x2