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Immunoglobulin evolution and associated factors with intestinal schistosomiasis after treatment in rural Burkina Faso

Nina Gouba[1]*, Hermann Sorgho[2], Isidore Traoré[3,4], Olivier O. Sombié[5], Yeri E. Hien[6], Nico F. W. Dena[1] and Jean B. Ouédraogo[5]

[1]Université Nazi Boni, Unité de Formation et de Recherche en Sciences et Techniques UFR/ST, Bobo Dioulasso 01 BP 1091, Burkina Faso.
[2]Institut de Recherche en Sciences de la Santé Direction Régionale Centre Ouest, Nanoro, BP 218, Burkina Faso.
[3]Centre Muraz, Bobo-Dioulasso, Institut National de Santé Publique, Bobo-Dioulasso, 01 BP 390, Burkina Faso.
[4]Université Nazi Boni, Institut Supérieur des Sciences de la Santé (IN.S.SA), Bobo-Dioulasso, 01 BP 1091, Burkina Faso.
[5]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo Dioulasso, 01 BP 545, Burkina Faso.
[6]Université Joseph Ki-Zerbo, Unité de Formation et de Recherche en Science de la Vie et de la Terre (UFR/ ST), Laboratoire de Biochimie et d'Immunologie Appliquée (LaBIA) 03 B. P 7021 Ouagadougou, Burkina Faso.

The performance of specific antibodies in a post-chemotherapy situation remains a controversial issue. Thus the study aims to investigate the variations of different immunoglobulins, IgG, IgG subclasses, IgM and IgA against *Schistosoma mansoni* soluble eggs antigens post-treatment and their correlation with eggs excretion. A longitudinal study was conducted on 928 participants over 5 years of age from February 2007 to March 2008 in "Vallée du Kou" in Burkina Faso, a rural area where *S. mansoni* is endemic. At the inclusion and during follow-up visits (post 1.5, 3, 6 and 12 months), stools and blood samples were examined for Schistosome eggs obtained from stools by the Kato-Katz test method and serum antibodies (IgM, IgA, IgG, IgG1, IgG2, IgG3, IgG4) obtained from blood by ELISA targeting the antigens of schistosome eggs. Subjects who were stool-egg-positive were treated with praziquantel in a single oral dose of 40 mg/kg. The overall prevalence of *S. mansoni* infection was 23.27% (216/928) with 77.32% (167/216) having a light intensity of infection. Factors associated with parasite eggs load were IgA, IgG1 and IgG4 titres. During each visit, each increase in IgA titre of 1 unit was associated with a significant reduction in parasite egg load of 50 eggs (p <0.002). The increase in IgG1 and IgG4 titres by 1 unit was associated with significant increases in the parasite egg count of 21 and 32, respectively. IgG1, IgG2 and IgG4 titres decreased significantly during the follow-up time (p<0.0001). In contrast, the titre of IgG3 increased significantly (p=<0.0001). This study showed that IgA, IgG1 and IgG4 are associated with decrease in parasite egg loads in stool and could be a good marker for treatment efficacy.

Key words: Immunoglobulins, *Schistosoma mansoni*, eggs, praziquantel, ELISA, kato-katz, Burkina Faso.

INTRODUCTION

Schistosomiasis remains a major neglected tropical Parasitic disease, particularly in Sub-Saharan Africa,

where around 80-90% of the cases are found (LoVerde, 2019). More than 240 million people are infected in 78 countries, especially in poor settings without access to safe drinking water and adequate sanitation (Steinmann et al., 2006; Colley et al., 2014). In Burkina Faso, *Schistosoma mansoni* and *S. haematobium* are present with variable levels of endemicity. But most of the reports on Schistosoma infections are related to urinary schistosomiasis (Poda et al., 2004). *S. mansoni* has been initially described in the Western and Southern parts of the country (Ouedraogo et al., 2016; Poda et al., 2004).

The current global strategy recommended by the World Health Organization (WHO) to control schistosomiasis is based on preventive chemotherapy or mass drug administration in at risk populations (Assaré et al., 2016; Fenwick and Jourdan, 2020). Since 2002, treatment based on Praziquantel (PZQ) was used for schistosomiasis in endemic countries in Africa. This treatment was supported by the Schistosomiasis Control Initiative Program (WHO, 2002). In Burkina Faso, the national program was focused on children in hyper-endemic areas (Ouedraogo et al., 2016).

Praziquantel (PZQ) treatment has been reported to enhance host protective immunity by exposing parasite antigens (Harnett and Kusel, 1986; Doenhoff et al., 2008). Thus, treatment allows the host immune systems to recognize *Schistosoma* adult worm antigens and to develop protective immunity (Mutapi et al., 2005). In addition, previous studies have shown tremendous variation in the changes in immune responses after chemotherapy between different human populations (Mutapi, 2001). *Schistosoma* specific antibodies appear to play an important role in this protective immunity after treatment. Schistosoma egg antigen-based tests have proven to be more sensitive than worm antigen-based tests (Mott et al., 1987; Hamilton et al., 1999). Their performance in a post-chemotherapy situation remains a subject of debate. Some studies have shown that antibodies against *Schistosoma* soluble eggs antigens (SEA) decline after treatment within few months (Rabello et al., 1997; Doenhoff et al., 2004; Hamilton et al., 1999). Here, we aim to monitor the different immunoglobulin IgG, IgG subclasses, IgM and IgA against SEA eggs and their correlation with egg excretion.

MATERIALS AND METHODS

Study area

The study was conducted in "Vallée du Kou", a locality where *S. mansoni* is endemic. The "Vallée du Kou" is a rural commune located 25 km from Bobo Dioulasso (Western Burkina Faso). The climate is Sudano-Sahelian. Kou's river runs through the valley, which is dominated by agricultural activities, with emphasis on rice production.

Study type and population

A longitudinal study was carried out from February 2007 to March 2008 involving individuals of both sexes aged 5 to 70 years living in the Kou Valley. Their willingness to participate was an important criterion of inclusion. We did an exhaustive sampling and included 928 participants. In addition, stool and blood samples were collected at every visit for parasitological analysis and an enzyme-linked immunosorbent assay test (ELISA test).

Stool and blood sample collections

At the moment of inclusion and during each follow-up visit (post 1.5, 3, 6 and 12 months), stool and blood samples were collected. Stool samples were collected in a sterile tube and stored at 4°C until parasitological examination as per Pawar et al. (2018). A volume of 5 mL of venous blood was collected from each participant during every visit. Serum was obtained from the blood samples by centrifugation (2000 rpm, 30 min at room temperature) aliquoted in sterile microtubes and stored at -20°C until the time of use.

Parasitological examination (microscopy)

Stool samples were analysed using the Kato-Katz test method (Katz and Pellegrino, 1972). Two slides for each stool sample were prepared using 42 mg of stool within 24 h. Stool microscopic examination and egg count were performed as previously described (Sorgho et al., 2005; Ouedraogo et al., 2016). The number of *S. mansoni* eggs per stool gram was obtained by multiplying by 24 the number of eggs counted per slide with a minor modification as previously described (Katz and Pellegrino, 1972). Eggs were counted and the intensity of the infection was expressed as the number of eggs per gram (epg) of faeces (Gupta and Singla, 2012).

Enzyme-linked immunosorbent assay (ELISA)

The ELISA test was used to detect the serum antibodies (IgG, IgM, IgA, IgG1, IgG2, IgG3 and IgG4) targeting the antigens of *S.mansoni* eggs. The ELISA test was performed as previously described (Sorgho et al., 2005). A soluble egg antigen (SEA) was obtained from a Puerto Rican strain of *S.mansoni* at Bangor, UK (Doenhoff et al., 2004). One hundred microliters of soluble antigens of *S. mansoni* diluted in carbonate–bicarbonate buffer, pH 9.6, at a concentration of 1 mg/ml were coated on each well of a polystyrene 96-well plate (Maxisorb; Nunc, Roskild, Denmark). The plates were incubated for 3 h at room temperature and overnight at 4°C. The following day, the plates were washed three times with a solution phosphate-buffered saline (PBS, 0.01M, pH 7.4) containing 0.05% Tween 20 (PBS-T), which was used for all washes. All plates were blocked with 200 µl/well PBS-T containing new-born calf serum (NCS; 5%) for 1 h at 37°C and washed three times. Serum (diluted

*Corresponding author. E-mail: goubanina@yahoo.fr. Tel: 00226 61395995.

Table 1. Sociodemographic characteristics of study participants.

Characteristics	Number	(%)
Age (age groups years)		
5-14	114	52.78
>14	102	47.22
Sex		
Female	118	54.63
Male	98	45.37
Intensity of infection (epg)		
1-99 (light)	167	77.31
100-399 (moderate)	43	19.91
≥400 (heavy)	6	2.78

1:100 in PBS-T-NCS) was added (100µl/well) into each well and incubated for 1 h at room temperature. After washing again with PBS-T, 100µl of peroxidase-conjugated goat anti-human IgG, (IgG, IgG1, IgG2, IgG3, IgG4, IgM and IgA,) (Southern Biotech) diluted to 1:1000 was added in each well for 2 h at 37°C and then washed three times. A volume of 100 µl of the substrate O-phenylenediamine (OPD; Sigma, Deisenhofen, Germany) with 3% H_2O_2 in 0.02M citric acid and 0.05 M sodium phosphate, pH 5.0 was added to each well. After 10 min of incubation in the dark, the reaction was stopped with 30 µl of H_2SO_4 (4N). The absorbance was then measured at 490 nm by using an ELISA plate reader (µQuant Biotech). For each immunoglobulin, the cut-off was defined as the mean OD (optical density) plus two standard deviations (S.D.) with 22 control sera from healthy German blood donors. The positive reactions were those with OD values above the respective cut-off values.

Patients' treatment

Subjects having positive *S. mansoni* eggs in stool samples using the Kato-Katz test method received a single dose of PZQ of 40 mg/kg and were followed up from February 2007 to March 2008 at 1.5, 3, 6 and 12 months post treatment. During the follow-up visits, all positive patients were treated with the same dose of PZQ.

Statistical analysis

Standard statistical methods and available-case analyses were used to describe variables using proportions with their number of subjects or mean with standard deviation (SD). We used a mixed model with linear logistic regression to identify the factors associated with parasite egg load evolution during the follow-up. For all statistical tests performed, a p-value of less than 0.05 was considered to be statistically significant. Data were analysed using SAS software, version 9.4.

Ethical consideration

Before starting data collection in the field, the study protocol was approved by the local ethics committee of the Centre Muraz under number N/Ref.016-2005/CE-CM. Participants were also asked to provide a written informed consent form to participate in the study. For children of less than 14 years old, parents or legal guardians signed the informed consent form.

RESULTS

Socio-demographic characteristics of the study participants

Among the 928 participants enrolled, 216 (23.27%) were positive for *S. mansoni* infection based on microscopy examination. Of the 216 participants with *S. mansoni* infection, 52.78% were children (5-14 years) and 47.22 % were adults (>14 years). The majority of the positive participants had a light infection (77.31 %) with egp <100 (Table 1).

Parasite change evolution after treatment during the follow-up time

At the inclusion, the mean number of eggs was 26 ± 2.11 and during follow-up, there was a reduction of about two eggs at each visit and this reduction was statistically significant (p=0.0006) (Figure 1).

Factors associated with parasite egg load evolution during the follow-up time

The only factor associated with the reduction of the parasite egg count during follow-up was a high IgA titre. Indeed, at each visit, each increase of 1 unit in IgA titre was associated with a significant reduction in the parasite egg load of 50 eggs (p = 0.002). IgG1 and IgG4 were
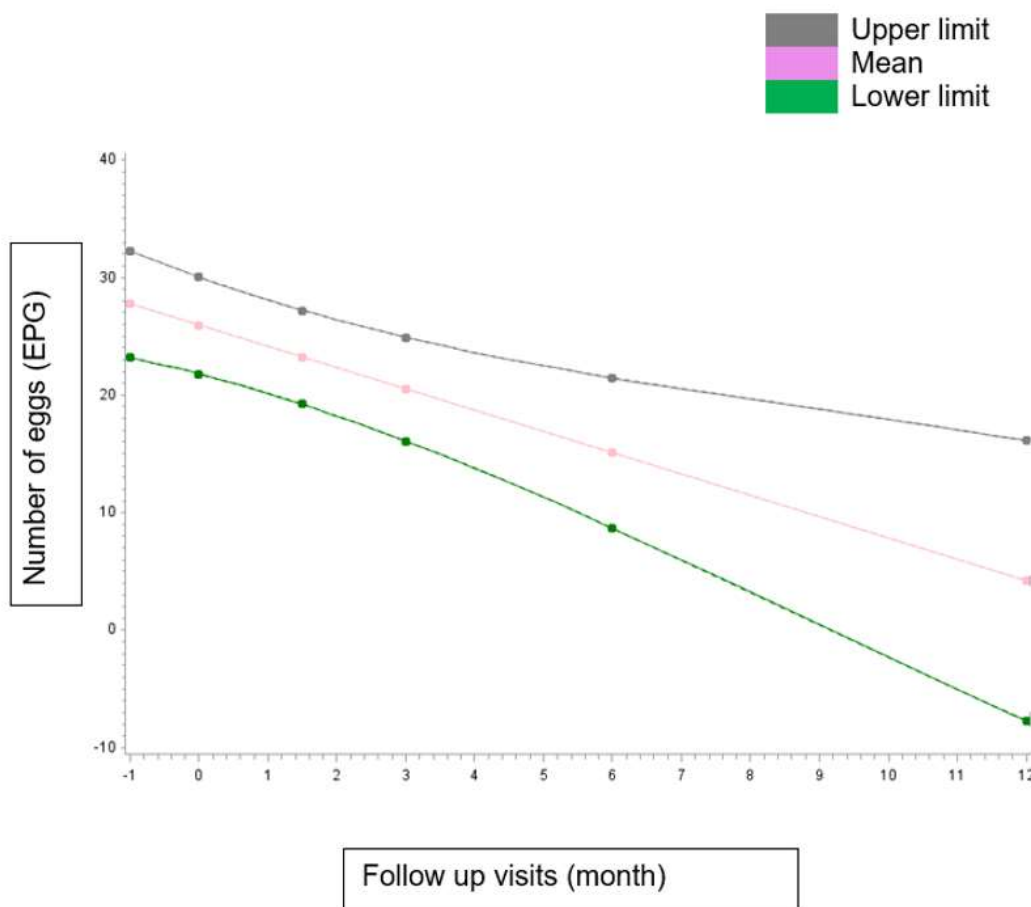
Figure 1. Evolution of parasite charge after treatment during follow up visits time.

Table 2. Mixed model: associated factors with the evolution of parasite eggs load during follow-up.

Parameter	Estimation (number of eggs)	P-value
Intercept (average number of eggs at enrollment.)	15.53	0.121
Slope (rise of the number of eggs over time)	-0.84	0.22
IgA (DO)	-50.36	0.002
IgG1(DO)	20.66	0.01
IgG4(DO)	32.41	<0.001
Ages (years)	-0.13	0.49

good markers of an increased parasite egg count. In fact, at each visit, the 1-unit increase in IgG1 and IgG4 titres was associated with a significant increase in the parasite egg count of 21 and 32, respectively (Table 2).

Immunoglobulins evolution after treatment during the follow-up time

The evolution of the different antibody titres during follow-up is shown in Figure 2. The immunoglobulins IgG, IgG1, IgG2 and IgG4 titres decreased significantly during the follow-up time (p<0.0001). In contrast, the titre of IgG3 increased significantly (p=<0.0001). The variation of IgA and IgM titres that was observed was not statically significant.

DISCUSSION

The infection intensity reported in our study included light
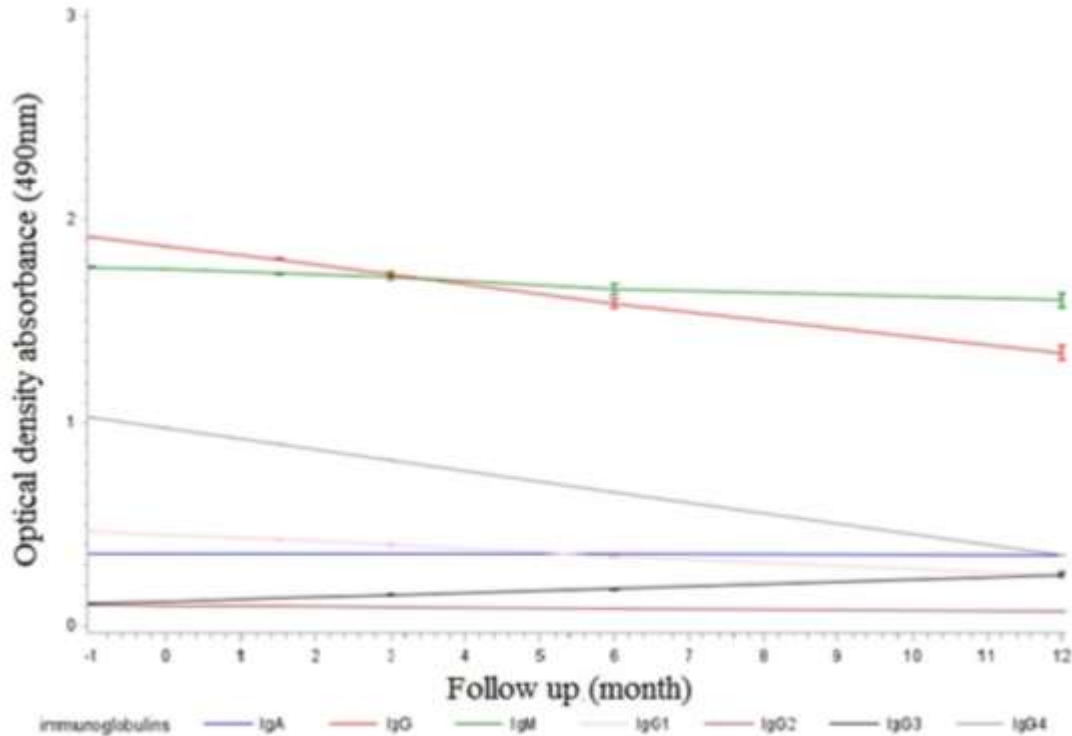
Figure 2. Immunoglobulins profile during the follow up visits.

infection levels for most participants, as previously reported, in the same locality (Sorgho et al., 2005). Our results showed a significant decrease in the parasite egg load during the follow-up time. This result was expected, supporting the well-documented effect of PZQ in the significant reduction in egg load after intervention and during follow-up (Reta and Erko, 2013; Bajiro et al., 2016).

The immunoglobulin IgA response led to a reduced egg number, whereas IgG1 and IgG4 titres increased in response to egg excretion. This observation is supported by a previous study that observed a tendency for *Schistosoma* whole worm antigen–IgA (anti-WWA-IgA) levels to be elevated (Fukushige et al., 2019). IgA response led to a significant reduction in the egg number during the follow-up. Some subjects might have been re-infected during the follow up, which could have induced the increased response of IgA. Indeed, the participants in this study work in rice fields, which explains the continuous exposure to re-infection. It has been shown that the immunoglobulin IgA response increases after *S. mansoni* egg deposition in the intestines (Poulain-godefroy et al., 1996).

In addition, the increase of IgG1 and IgG4 titres is found to be associated with significant increases in the egg number in this study, which might be related to the presence of egg excretion by the parasite. The increased titres of IgG1 and IgG4 could be a response against re-infection after treatment. In addition, PZQ kills only mature worms, so immature worms may escape treatment. Thus, the time interval between each monitoring period could allow the immature worms to mature and explain the excretion of eggs or reinfection. Previous studies have shown that IgG4 response is associated with higher parasite burdens and susceptibility to reinfection post-treatment (Mutapi, 2001; Satti et al., 1996).

Regarding the evolution on antibody response, the significantly decreased level of IgG, IgG1, IgG2 and IgG4 titres during the follow-up time is associated with the reduction of parasite egg excretion after treatment. Similar observations have been reported previously (Fukushige et al., 2019). In contrast, IgG3 titres increased after treatment during the follow-up. This result is similar to a previous observation in one year post-treatment (Abebe et al., 2001). Immunoglobulin IgG3 response was observed to kill schistosomula in the presence of activated eosinophil (Chisango et al., 2019). The IgG3 responses could be a response to the parasite stage of those that escaped the PZQ treatment. The main limitation of our study was the loss of follow up during each follow up visit. Although, to our knowledge no

further study has been carried out before now on the study area despite the fact that the data had been collected for a long time. Thus, data might be some indication of schistosomiasis situation in the study area.

Conclusion

Praziquantel treatment reduced the *S. mansoni* egg load one year post-treatment. Immune responses are potentials factors associated with a significant reduction in the parasite egg load. The titres of IgA, IgG1 and IgG4 are found to be associated with parasite egg load and could be a good marker for treatment efficacy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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