

## **Seroprevalence to Schistosoma Soluble Egg Antigen among Nomadic Pastoralists Residing in Northern Senegal**

Authors: Seck, Mame Cheikh, Badiane, Aida Sadikh, Thwing, Julie, Ndiaye, Mouhamadou, Diongue, Khadim, et al.

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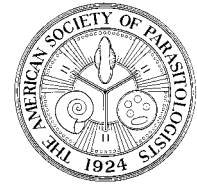
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## SEROPREVALENCE TO SCHISTOSOMA SOLUBLE EGG ANTIGEN AMONG NOMADIC PASTORALISTS RESIDING IN NORTHERN SENEGAL

Mame Cheikh Seck<sup>1,2</sup>, Aida Sadikh Badiane<sup>1,2</sup>, Julie Thwing<sup>3</sup>, Mouhamadou Ndiaye<sup>1,2</sup>, Khadim Diongue<sup>1,2</sup>, Ibrahima Mbaye Ndiaye<sup>2</sup>, Mamadou Alpha Diallo<sup>1,2</sup>, Mohamed Sy<sup>2</sup>, Jules François Gomis<sup>2</sup>, Tolla Ndiaye<sup>2</sup>, Aminata Gaye<sup>2</sup>, Yeuk-Mui Lee<sup>3</sup>, W. Evan Secor<sup>3</sup>, Daouda Ndiaye<sup>1,2</sup>, and Eric Rogier<sup>4,5</sup>

<sup>1</sup> Department of Parasitology, Faculty of Medicine and Pharmacy, Cheikh Anta Diop University, Dakar 16477, Senegal.

<sup>2</sup> International Research & Training Center in Applied Genomics and Health Surveillance (CIGASS), Cheikh Anta Diop University, Dakar 16477, Senegal.

<sup>3</sup> Division of Parasitic Diseases and Malaria, U.S. Centers for Disease Control and Prevention, Atlanta, Georgia 30329.

<sup>4</sup> Current address: Division of Digestive Disease and Nutrition, University of Kentucky, Lexington, Kentucky 40506.

<sup>5</sup> At the time of this work was employed by the U.S. Centers for Disease Control and Prevention, Atlanta, Georgia 30329.

Correspondence should be sent to Mame Cheikh Seck (<https://orcid.org/0000-0002-8326-5359>) at: [mamecheikh.seck@ucad.edu.sn](mailto:mamecheikh.seck@ucad.edu.sn)

### KEY WORDS ABSTRACT

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Urinary and intestinal schistosomiasis are endemic in Senegal, with prevalence heterogeneous throughout the country. Because of their way of life, nomadic pastoralists are not typically included in epidemiological surveys, and data on the prevalence of schistosomiasis in Senegalese nomadic populations are largely non-existent. The purpose of this study was to determine the seroprevalence of schistosomiasis in Senegalese nomadic pastoralists. A modified snowball sampling survey was conducted among 1,467 nomadic pastoralists aged 6 mo and older in 5 districts in northern Senegal. Dried blood spots from participants of all ages and data regarding demographics were collected to assess IgG antibody responses against *Schistosoma mansoni* soluble egg antigen (SEA) using a bead-based multiplex assay. Out of 1,467 study subjects, 1,464 (99.8%) provided IgG serological data that cleared quality assurance. Of the participants with appropriate data, 56.6% were male, the median age was 22 yr, and 31.6% were under 15 yr of age. The overall anti-SEA IgG seroprevalence was 19.1% (95% confidence interval [CI]: 17.1–21.1%) with the highest estimates observed in Dagana (35.9%) and the lowest observed in Podor nomadic groups (3.4%). Antibody responses increased significantly with age except for the oldest age groups (>40 yr of age), which saw lower levels of antibody response compared to younger adults. When controlling for age and location by multivariate regression, the male sex was associated with a 2-fold greater odds of anti-SEA IgG seropositivity (aPOR: 2.0; 95% CI: 1.5–2.7). Serosurveys for anti-SEA IgG among nomadic peoples in northern Senegal found a substantial percentage of individuals with evidence for current or previous *Schistosoma* spp. infection with the highest levels of exposure in the district adjacent to the Diama dam along the Senegal River. With IgG prevalence increased by age except in the older adults, and the male sex significantly associated with seropositivity, these data point toward sex-associated behavioral practices and human environmental modification as risk factors for *Schistosoma* exposure.

Human schistosomiasis, also known as bilharziasis, is one of the most important neglected tropical diseases (NTDs) (WHO, 2012). It is a parasitic disease caused by flukes (trematodes) of the genus *Schistosoma*. It is widespread, ranking as the third most detrimental parasitic disease after malaria and intestinal helminthiasis in terms of socio-economic and public health significance in tropical and subtropical areas. According to the World Health Organization, a total of 78 countries have ever reported endemic schistosomiasis, and it is currently endemic in 52 countries. The World Health Organization (WHO) Global Health Estimates from 2016 projected that with a death rate of 0.3 per 100,000, schistosomiasis was responsible for 24,000 deaths in 2016, a decrease from 55,000 in 2000 (Lackey and Horrall, 2022).

Schistosomiasis has a considerable impact on the health of populations, especially schoolchildren, adolescents, and adults with a profession or activity involving contact with fresh water (such as farmers, irrigation workers, and women in domestic work) (Gryseels et al., 2006). In Senegal, like all of Africa, *Schistosoma haematobium* and *Schistosoma mansoni* are the predominant species causing disease, with prevalence unevenly distributed throughout the country and often overlapping (Ndir, 2000). *Schistosoma haematobium* infection, which causes urogenital schistosomiasis, is prevalent in all regions, while *S. mansoni* infection, which causes intestinal schistosomiasis, predominates in the northern districts. The construction of the Diama dam (and

the Manantali dam in Mali) in the 1980s significantly altered the ecology of the Senegal River and contributed to the clinical recognition of a large increase in the prevalence of intestinal schistosomiasis (Talla et al., 1990). National control efforts began with a prevalence survey in 1996 followed by a national control program backed by the World Bank in 1997 (Ndir, 2000). According to the preventative chemotherapy databank provided by the WHO, 4 large-scale attempts have targeted curbing schistosomiasis in Senegal. Including the smaller 2009 attempt with the larger 2010, 2012, and 2013 ones, 3.3%, 14.2%, 29%, and 42% of the total population was treated, respectively, focused primarily on school-aged children (Sokolow et al., 2015). The major activity of this program is mass drug administration (MDA) mainly among school children according to the WHO recommendations after prevalence mapping.

Disease mapping and post-MDA monitoring of the infection is based on microscopy techniques to detect parasite eggs. In the field, the most widely used diagnostic assays are urine filtration for urogenital schistosomiasis and the Kato-Katz technique for diagnosing intestinal schistosomiasis. However, in individuals with low infection intensities or low endemic areas, egg detection techniques are less sensitive. Over the last decades, various serological methods have been developed to detect antibodies against schistosome antigens. Different techniques have been applied, including indirect immunofluorescent antibody tests (IFATs), indirect hemagglutination assays (IHAs), and enzyme-linked immunosorbent assays (ELISAs) using different antigens, such as crude or purified adult worm antigen (AWA), schistosome soluble egg antigen (SEA), and cercarial antigen (CA) preparations (Nash, 1978; Ambroise-Thomas and Grillot, 1980; Ambroise-Thomas et al., 1981; Deelder and Cornelis, 1981; Mott and Dixon, 1982; Hamilton et al., 1999; Tarp et al., 2000; Whitty et al., 2000; van Gool et al., 2002; Doenhoff et al., 2004; Bierman et al., 2006; Chand et al., 2010; Porte et al., 2020). A limitation of serological assays is that using current antigens, they are unable to discriminate between active infection and past exposure. However, they are clinically useful for estimating prevalence among untreated endemic populations as well as diagnosing symptomatic travelers who are not from an endemic area. Newly developed multiplex bead assays (MBAs) that can simultaneously detect antibodies to multiple antigens could make it possible to gather data on several diseases with limited sample volumes (Lammie et al., 2012).

Senegal hosts a population of nomadic pastoralists who lead their herds to the south in search of pasture during the dry season and spend the rainy season in the northern districts. These mobile populations and their families—often considered as ‘hidden or hard to reach’ individuals—face complex obstacles in accessing essential health care and education. Recently, a study demonstrated that the majority of the Senegalese nomadic pastoralists are illiterate; only 4% could read and understand French, and 2% could read and understand their language in Arabic script (Seck et al., 2017). These findings and their way of life strongly indicate that nomadic children do not attend school and are not included in epidemiological surveys by the national schistosomiasis control program. Consequently, data on schistosomiasis in Senegalese nomadic populations are largely non-existent. This current study aimed to collect information on anti-SEA IgG antibody levels among nomadic pastoralists, to compare relative seroprevalence levels among age groups, sex, and enrollment districts, and to better understand whether schistosomiasis may be a health issue in need of attention in this population.

## MATERIALS AND METHODS

### Study areas

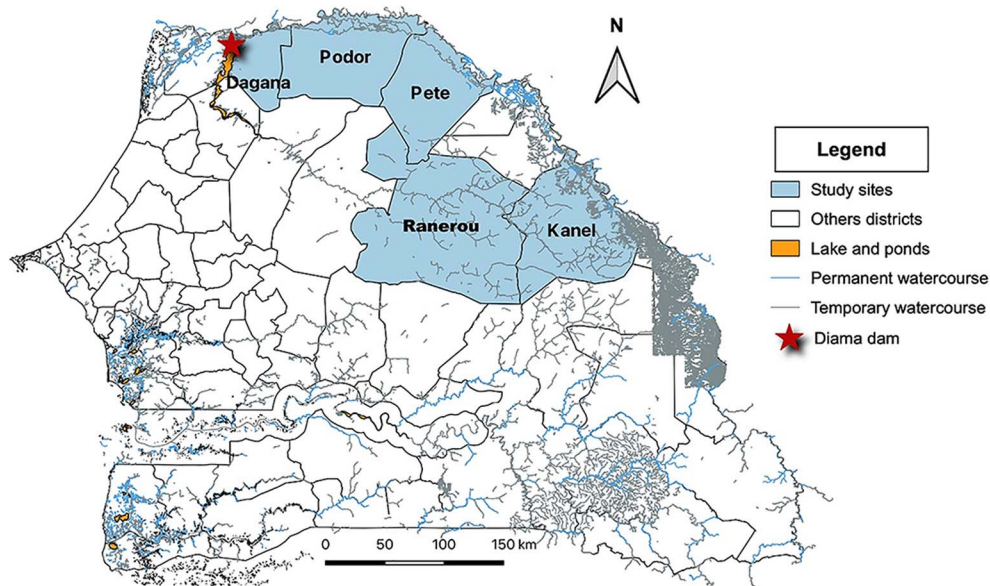
As described previously (Seck et al., 2017), the original study was focused on malaria prevalence estimates in this population, but the collection of blood samples was utilized for serological data collection of multiple endemic infectious diseases. The survey was carried out from September to October 2014 in 2 areas in northern Senegal: the Senegal River valley and the Ferlo desert. In these areas, the annual rainfall can reach 600 mm, increasing from north to south. These sites are known to host large numbers of nomadic pastoralists who do not have access to tap water and therefore may be at risk of schistosomiasis. For this analysis, 5 districts were included and selected as the study sites as they are known to host large numbers of nomadic pastoralists during the rainy season (primarily because of the presence of water sources for their livestock). Three of the selected sites were in the Senegal River Valley, in the health districts of Dagana (16°30′38.027″N, 15°39′12.524″W), Podor (16°39′36.212″N 14°57′33.584″W), and Pete (16°10′67.359″N, 13°94′73.454″W). The Ferlo desert is a vast Sahelian plain of more than 75,000 km<sup>2</sup> located along the Senegal River in northeastern Senegal. Temporary ponds and backwaters are formed during the rainy season between July and October. These fresh waters are popular for domestic activities and livestock watering. In this study area, 2 districts were chosen: Ranerou (15°29′80.916″N, 13°96′22.850″W) and Kanel (15°49′10.26″N, 13°17′56.84″W) (Fig. 1).

### Study design, data, and sample collection

Written informed consent was obtained from all study participants before they were enrolled in the study. For children <15 yr of age informed consent was provided by their parents or legal guardian. Participants aged between 15 and 17 yr gave their assent. The details of the study design have been described elsewhere (Seck et al., 2019a). Briefly, a snowball sampling survey using a modified respondent-driven sampling (RDS) methodology was conducted. After informed consent, a questionnaire was administered for collecting socio-demographic data (age, gender, and ethnicity). For each participant, 3 to 4 drops of blood were collected on Whatman 903 protein saver card (Sigma-Aldrich, St. Louis, Missouri) filter paper, dried at room temperature, sealed in plastic bags with silica gel desiccant, and stored at −20 °C until serological testing. The survey was approved by the ethical review committee of the Ministry of Health, Senegal (Approval 324/MSAS/DPRS/CNERS). The activity was considered a public health program activity and not human subjects research by the Center for Global Health of Centers for Disease Control and Prevention Human Subjects Office (no. 2014-193a).

### SEA-IgG antibody testing by bead-based multiplex IgG detection assay

Magnetic microbeads (Luminex Corp., Austin, Texas) were coupled with 120 µg *S. mansoni* SEA per 1 ml of beads in PBS. Sera from persons with either *S. haematobium* or *S. mansoni* infections are reactive with this antigen preparation. A 6 mm circular punch was taken from the center of each blood spot, corresponding to 10 µl whole blood, for sample elution. Samples were diluted in blocking Buffer B (phosphate-buffered saline [PBS



**Figure 1.** Map of northern Senegal with study sites for enrollment (light blue). Diama dam, lake, and ponds are located in the Dagana district. Temporary watercourses are more frequent in Ranerou and Kanel districts.

pH7.2]), 0.5% polyvinyl alcohol (Sigma-Aldrich), 0.8% polyvinyl pyrrolidine (Sigma-Aldrich), 0.1% casein (ThermoFisher, Waltham, Massachusetts), 0.5% BSA (Millipore, Burlington, Massachusetts), 0.3% Tween-20, 0.1% sodium azide, and 3 µg/ml *Escherichia coli* extract to prevent non-specific binding and stored at 4 °C until analysis.

Bead-based multiplex technology was used as described previously (Seck et al., 2020). Briefly, in 5 ml reagent buffer (Buffer A: PBS, 0.5% BSA, 0.05% Tween-20, 0.02% NaN<sub>3</sub>), a bead mix was prepared with all antigen-conjugated regions included, and 50 µl bead mix was pipetted into each well of a BioPlex Pro plate (BioRad, Hercules, California). Beads were washed 2× with 100 µl PBS, 0.05% Tween-20 (PBST) and 50 µl reagent mix (in 5 ml Buffer A: 1:500 anti-human IgG [Southern Biotech, Birmingham, Alabama], 1:625 anti-human IgG<sub>4</sub> [Southern Biotech]); 1:200 streptavidin-PE (Invitrogen, Waltham, Massachusetts) was added to all wells; 50 µl of samples (or controls) was then added to the appropriate wells at a sample dilution of 1:50. Plates were incubated overnight with gentle shaking at room temperature and protected from light. The next morning (after ~16 hr total incubation time), plates were washed 3 times with PBST, and beads were resuspended with 100 µl PBS and read on a MAGPIX machine (Luminex Corp.). The MFI signal was generated for a minimum of 50 beads/region, and background median fluorescence intensity (MFI) from wells incubated with Buffer B was subtracted from each sample to give a final value of MFI-bg.

Antigens are expressed as glutathione-S-transferase (GST)-tagged proteins, with GST-coupled beads included as a control. Samples with high MFI-bg values for GST (>100) were considered to have potential evidence of non-specific binding and were removed from serological analyses.

### Statistical analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, North Carolina). A seropositivity threshold was determined

by a panel of 91 sera from U.S. residents without a history of international travel. The mean + 3 standard deviations of the MFI-bg signal from this sample set was used as the seropositivity threshold with any study blood samples with an MFI-bg signal above this threshold being categorized as IgG seropositive (Suppl. Fig. S1). For risk factor analysis and estimates of prevalence ratios (PORs), univariate and multivariate logistic regression was performed in SAS under the PROC LOGISTIC procedure. The relationship between SEA IgG positivity and age was modeled with local polynomial (LOESS) regression performed with the PROC SGPLOT procedure with cubic interpolation (degree of 1) and a smoothing parameter of 1.0.

### RESULTS

For this study, 1,467 nomadic pastoralists were enrolled in 5 districts (Fig. 1). As quality assurance for the SEA IgG assay, any blood samples with evidence of non-specific binding (as described in Methods) were removed from further analyses. Three blood samples (0.2%) were thus excluded, resulting in a final sample set of 1,464 for subsequent serological analyses. From this population, approximately equivalent numbers of individuals were enrolled from each of the 5 districts in Senegal: Podor, Dagana, Kanel, Pete, and Ranerou (Table I). More males were enrolled ( $n = 828$ , 56.6%) than females ( $n = 636$ , 43.4%). The ages of participants ranged from 6 mo of age to 80 yr with a median age of 22 yr.

The sampling location was strongly associated with SEA IgG seropositivity. Seroprevalence estimates ranged from 3.4% (95% CI: 1.3–5.4%) in the Podor district to 35.9% (30.5–41.4%) in the Dagana district (Table I). Using the Dagana district as the referent due to the highest SEA seroprevalence, all other districts had significantly lower proportions of seropositive participants. Univariate regression found male sex was significantly associated with SEA seropositivity (POR: 1.5; 95% CI: 1.1–1.9). When compared to the youngest study participants (0–4 yr olds), all older



**Table I.** Number of participants, seropositivity status with soluble egg antigen (SEA) IgG antibodies, and crude and adjusted prevalence ratios by different variables: nomadic pastoralists in northern Senegal, 2014.

Category	No. enrolled with serology data	No. SEA seropositive (% seroprevalence)	Crude prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)
District				
Dagana	295	106 (35.9%)	1.0 (referent)	1.0 (referent)
Podor	296	10 (3.4%)	0.09 (0.06–0.14)***	0.08 (0.05–0.13)***
Kanel	293	40 (13.7%)	0.34 (0.24–0.49)***	0.27 (0.19–0.39)***
Pete	290	56 (19.3%)	0.42 (0.30–0.59)***	0.40 (0.28–0.56)***
Ranerou	290	67 (23.1%)	0.59 (0.43–0.82)**	0.61 (0.44–0.85)**
Gender				
Female	636	100 (15.7%)	1.0 (referent)	1.0 (referent)
Male	828	179 (21.6%)	1.5 (1.1–1.9)**	2.0 (1.5–2.7)***
Age				
0–4	111	3 (2.7%)	1.0 (referent)	1.0 (referent)
5–10	218	41 (18.8%)	8.3 (2.5–27.6)***	4.4 (2.1–9.1)***
11–15	172	36 (20.9%)	9.5 (2.9–31.8)***	6.6 (3.2–13.8)***
16–20	190	40 (21.0%)	9.6 (2.9–31.8)***	6.3 (3.0–13.1)***
21–30	295	63 (21.4%)	9.8 (3.0–31.8)***	8.1 (4.0–16.5)***
31–40	187	46 (24.6%)	11.7 (3.6–38.8)***	8.2 (4.0–17.0)***
41–50	133	32 (24.1%)	11.4 (3.4–38.4)***	5.9 (2.8–12.7)***
>50	158	18 (11.4%)	4.6 (1.3–16.1)*	3.3 (1.5–7.0)**

Wald chi square test for statistical significance: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

age categories had a significantly higher percentage of enrolled participants who were seropositive by univariate regression. This trend of increasing seropositivity by age categories held true until the 41–50-yr group, which showed a slightly reduced seroprevalence compared to the 31–40 yr old group. The >50-yr group showed a 2-fold lower prevalence of participants who were seropositive compared to the peak prevalence.

Seropositivity estimates by age category increased until approximately 30 yr of age, whereas estimates then steadily decreased in a monotonic fashion for all older ages (Fig. 2A). This trend generally held consistent for participants enrolled within the different districts with a visual inflection point in seropositivity by age between 20 and 30 yr (Fig. 2). The exception to this was the Pete district with an inflection point around 50 yr of age. The absolute level of IgG antibodies against SEA rapidly increased within increasing age in the newborn to 15-yr old age categories (Fig. 2B). The IgG level appeared to be highest in the younger adults (ages 16–40) before showing lower estimates in older age categories, but with a high degree of variance. The regression model for seropositive proportion by age for those 28 yr and younger provided a positive linear slope of 0.009 (95% CI: 0.005, 0.013) with a  $P$  value  $< 0.0001$ . For persons older than 28 yr of age, the regression slope was  $-0.004$  ( $-0.006$ ,  $-0.001$ ) with a  $P$  value of 0.004.

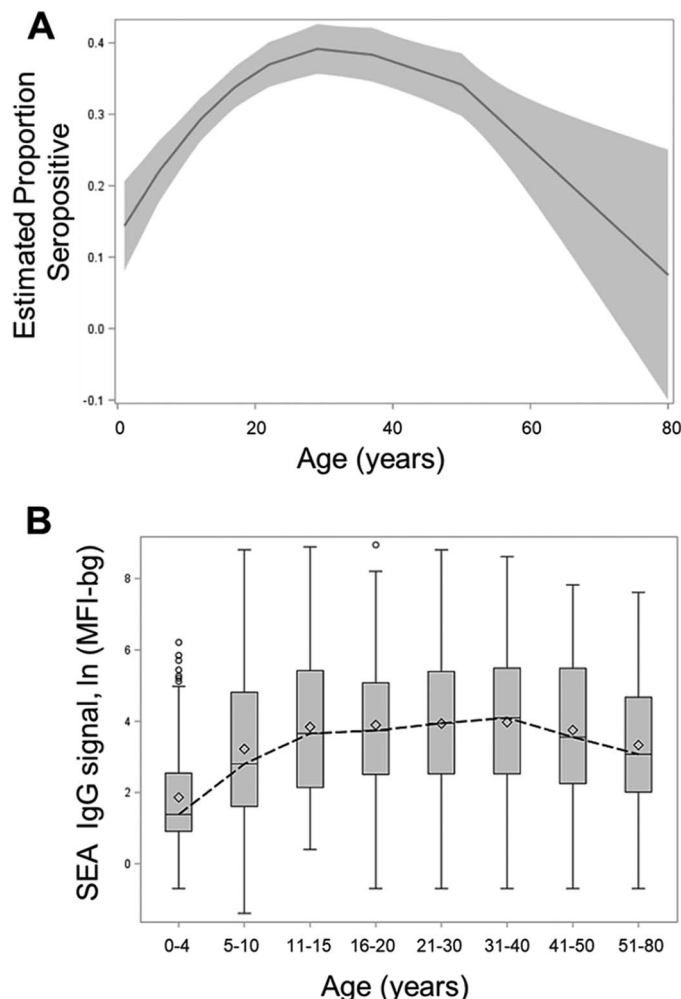
Multivariate logistic regression including age, sex, and district sampled found both sex and site of sampling were significantly associated with seropositivity to SEA, but no overall association with age. If the oldest age category of those over 50 yr was removed from the multivariate analysis, increasing age became significantly associated with higher seropositivity. The overall associations and statistical significance between crude and adjusted prevalence ratio estimates were largely unchanged (Table I), though compared to the referent of newborns to 4 yr olds, adjusted prevalence ratios were reduced for all the older age categories when compared to the crude estimates. When controlling for age and district of enrollment, the adjusted odds of SEA seropositivity were twice

as high for males compared to females (aPR: 2.0; 95% CI: 1.5–2.7). If assessing seropositivity dynamics by age and sex, persons aged newborn to 15 yr showed similar seropositivity regardless of sex, but male seropositivity remained higher for ages 15 to 60 with significantly higher differences from approximately age 35 to 50 compared to females (Fig. 3).

## DISCUSSION

The present study provides strong serological evidence of schistosome infections in nomadic populations in northern Senegal. As this survey of the nomadic population was a modified respondent-driven methodology (similar to snowball sampling), caution should be given in interpreting absolute seroprevalence levels as this sampling design was not meant to provide precise estimates. This current study focuses on relative differences among 3 variables of participants enrolled in this survey: age, sex, and district of enrollment. Senegal has one of the highest schistosomiasis disease burdens in the world with both *S. mansoni* and *S. haematobium* endemic throughout the country (Senghor, 2010). In 2000, nearly 8.5 million people were at risk of infection, and an estimated 1.3 million people were infected (Chitsulo et al., 2000). Though national control efforts focused on school-age children in epidemiological studies and mass drug treatment (Faust et al., 2020), Senegalese nomadic pastoralists infrequently attend school (Seck et al., 2017); thus, schistosomiasis prevalence data among nomadic people are not readily available, and these individuals are unlikely to benefit from associated public health initiatives.

IgG binding to SEA antigen is indicative of current or past exposure to schistosome parasites. The overall seroprevalence was 19.1% (95% confidence interval [CI]: 17.1–21.1%), and the presence of seropositivity among all age groups indicates that nomadic pastoralists can be exposed to schistosome infection throughout their lives. A strong association between SEA seroprevalence and district was observed. The highest seroprevalence



**Figure 2.** Seropositivity for IgG against soluble egg antigen (SEA) by age. (A) The estimated proportion of the nomadic pastoralist enrolled participants seropositive for anti-SEA IgG by age. The solid black line shows local polynomial (LOESS) regression with shading representing a 95% confidence limit. (B) Log-transformed assay signal for anti-SEA IgG by different age categories. Boxes display interquartile range (IQR) with whiskers extended  $1.5 \times$  IQR and circles as observations outside of  $1.5 \times$  IQR. Within each box, the diamond represents the mean, and the horizontal line shows the median. The hashed line between boxes connects medians.

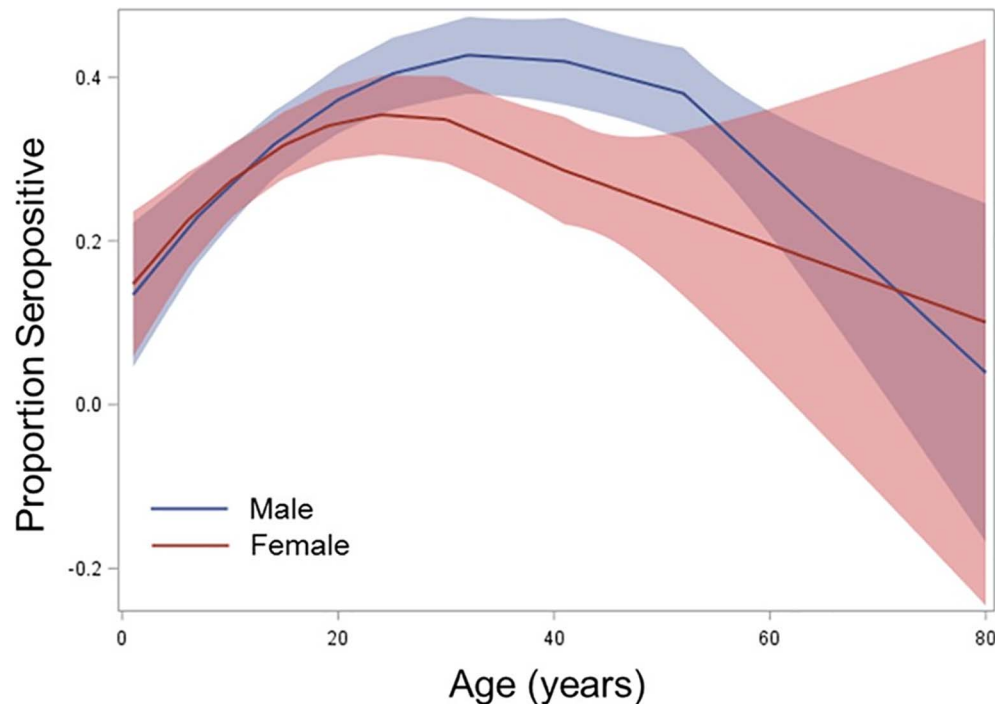
was found in Dagana, which may be explained by the fact that this district is located along the Senegal River basin where the Diama Dam was constructed in the 1980s, an event that significantly altered the ecology and contributed to a drastic increase in the intestinal form of schistosomiasis (Talla et al., 1990). Likewise, an increase in urogenital schistosomiasis has been noted in all ecological zones of the basin (Southgate, 1997; Urbani et al., 1997; Savaya Alkalay et al., 2014). The association between dams and human schistosomiasis has been recognized since the early 20th century, even though the mechanisms behind the association remain unclear. One intriguing hypothesis for the schistosomiasis outbreak in the Senegal River Basin is that the Diama Dam blocks the passage of native, migratory snail predators, notably *Macrobrachium* spp. river prawns from upstream locations. This released snail populations from predation pressure, resulting in increased numbers of snails

and the force of schistosomiasis transmission (Savaya Alkalay et al., 2014; Sokolow et al., 2014; Munoz, 2015). In a test of this hypothesis, a demonstration intervention in Senegal showed that restoring native *Macrobrachium vollohovenii* prawns to 1 village water-access point led to significantly lower human reinfection rates for schistosomiasis compared to a nearby control village (Sokolow et al., 2015). Interestingly, Dagana district residents born after Diama Dam was built (0–28 yr old) were found to have higher estimates of seropositivity to SEA as age increased, indicating a greater likelihood of exposure as persons have aged since dam construction. By contrast, seropositivity estimates monotonically became lower when age groups in Dagana were beyond 28 yr old. The age seroprevalence curves for persons sampled in the other districts that were less likely to have been affected by the construction of the dam demonstrated distinct patterns. However, these observations merit caution considering the nomadic characteristics of the study population.

With respect to sex, when controlling for age and district of enrollment, the adjusted odds of SEA seropositivity were twice as high for males compared to females, primarily driven by substantially higher seroprevalence in the 35-to-50-yr-old males. The reason for higher rates of male seroprevalence may be associated with greater exposure to infected snail-infested water bodies in their daily activities such as fishing and herding livestock by bodies of water. Males were also more prone to schistosome infections in other reports from Senegal (Thiam, 1993; Ka, 2002; Seck et al., 2019b) and in other countries in Africa (El-Gendy et al., 1999; Satayathum et al., 2006; Rudge et al., 2008; Kapito-Tembo et al., 2009; Ugbomoiko et al., 2010). Similarly, males presented a 3.39 (95% CI: 2.2–5.3) greater odds of *S. mansoni* infection in a study in Brazil ( $n = 858$ ) (Enk et al., 2010). However, these results do not agree with reports by Opara et al. (2007) in Nigeria, Dabo et al. (2011) in Mali, and Ahmed et al. (2012) in central Sudan who found a similar prevalence in males and females.

Limitations to this study include the wide range of distances traveled by pastoralists during wet and dry seasons, so it is difficult to ascertain where parasite exposure occurred. Additionally, the SEA preparation used is recognized by IgG antibodies from persons with either *S. mansoni* or *S. haematobium* infections, making the determination of species-specific exposure impossible to assess. Data were collected using a modified RDS approach, and the analysis was not weighted or adjusted to take the sampling method into account; thus the results may not be generalizable beyond the study participants. With sampling occurring in 2014, serological estimates for SEA could have changed since that time. Analytical methods used in this analysis were not designed for RDS data and may underestimate the true sampling variability in the data. Data methods were developed and collected for the primary study of malaria prevalence in these nomadic populations with a different purpose and may not be optimized for this analysis.

In summary, this study found through serology data a considerable proportion of nomadic pastoralists in northern Senegal had evidence for *Schistosoma* spp. exposure. Whether populations were sampled in districts adjacent to the Senegal River or more desert-like settings, data suggest that infection is widespread. Employment of the SEA target in serological studies is useful to provide information about schistosomiasis exposure in human populations.



**Figure 3.** Seroprevalence to soluble egg antigen (SEA) by sex and year of age. The proportion of the nomadic pastoralist enrolled participants seropositive for anti-SEA IgG by age and male (blue line) or female (red line) sex. Solid lines show local polynomial (LOESS) regression with shading representing a 95% confidence limit.

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