High Prevalence of Urinary Schistosomiasis in a Desert Population: Results from an Exploratory Study Around the Ounianga Lakes in Chad

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High prevalence of urinary schistosomiasis in a desert population: results from an exploratory study around the Ounianga lakes in Chad

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ABSTRACT

Background: Researching a water-borne disease in the middle of the Sahara desert might not seem the most relevant concern. However, nomadic Sahelian pastoralist's health concerns regarding their livestock and anecdotal reports about trematode infections of *Fasciola* spp and *Schistosoma* spp in desert-raised animals justified an exploratory study focusing on the lakes of Ounianga in Northern Chad. The aim was to test whether trematode parasites such as *Schistosoma* spp occur in human populations living around the Sahara desert lakes of Ounianga Kebir and Ounianga Serir in northern Chad.

Methods: The study comprised of three components. First, a cross sectional survey based on a random sample drawn from the population to detect infections with *S. haematobium* and *S. mansoni*; second, focus group discussions exploring disease priorities, access to health and health seeking behaviour; and third, searching water contact sites for intermediate host snails. Samples of trematode parasites and snails were confirmed on species level by molecular genetics methods.

Results: Among 258 participants, the overall *S. haematobium* prevalence using urine filtration was 39.1% (95% CI 33.2% – 45.1%), with 51.5% of the infected suffering from heavy infection. The intermediate host snail of *S. haematobium* (*Bulinus truncatus*) occurred at water sites near both study villages, revealing the potential for local transmission. Although a positive *S. mansoni* POC-CCA test result was obtained from 15.2% (10.6%-19.7%) of the samples no intermediate host snails of *S. mansoni* were found, and the relevance of *S. mansoni* remains uncertain. Qualitative findings underline the importance of morbidity caused by urinary schistosomiasis, and the lack of access to diagnostics and treatment as a major health concern.

Conclusion: This research revealed a high prevalence of urinary schistosomiasis in the population living around the lakes of Ounianga in the Sahara, a UNESCO world heritage site in Chad. Despite the high public health importance of the associated morbidity expressed by the population there is no access to diagnostics and treatment. Further research is needed to develop and test a context adapted intervention.

Key words

Bulinus truncatus. Chad, malacology, Ounianga, POC-CCA, prevalence, Sahara, *Schistosoma bovis, Schistosoma haematobium*, schistosomiasis

BACKGROUND

Schistosome infections are listed among the 20 neglected tropical diseases (NTDs) targeted by the World Health Organisation (WHO) for elimination by 2030 (1). In endemic regions, those populations affected by schistosomiasis are often those living in poverty and / or in settings with restricted access to clean water for their sanitation and hygiene needs (2). Worldwide, an estimated 230 million people harbour an infection with *Schistosoma* spp (3). Occupational and recreational activities in close contact with freshwater, e.g. fishing, doing laundry and bathing present the main risk of infections. Highest prevalence is commonly observed among school-aged children as they enjoy playing in stagnant water sites. Undetected and therewith untreated urinary or intestinal schistosomiasis leads to chronic infections and serious morbidities including a wide range of different pathologies as e.g. anaemia, stunted growth, impaired cognition and organ damages, that negatively affect economic activities and therewith maintain poverty (4, 5). The safe and effective drug, Praziquantel, is currently used for mass drug administration programs in endemic settings as well as for treatment of individual acute infections . However, its effectiveness is threatened by increasing resistance of the parasite that is observed (6).

The majority of schistosomiasis cases occur in sub-Saharan Africa, and the disease is reported from countries throughout the Sahel, including Mauretania, Mali, Niger, Chad and Sudan (7-11). Infections are predominantly due to *Schistosoma haematobium* which has the ability to maintain its life cycle in a semi-arid environment, including in the ecoregion of the Sahel (12). Yet, there are old reports on schistosomiasis occurrence also more to the north, from within the Sahara desert (7, 13). The occurrence of *Schistosoma* spp, a genus of water-transmitted parasites belonging to the clades of digenean trematodes, and its occurrence in the hot and hyper-arid desert may seem surprising but occurrence in at least two desert-specific ecosystems have been described so far. These are (a) oases where schistosomiasis transmission is linked to man-made irrigation systems (14, 15), and (b) areas with reclaimed land for agriculture, made cultivable by artificial irrigation from deep wells (16).

Anecdotal reports from nomadic Sahelian pastoralists on *Fasciola* spp, another digenean trematode species, in livestock raised in the Chadian Sahara and recent reports about modern and early Holocene finding of intermediate host snails pointed towards the occurrence and potential ongoing transmission of schistosomiasis at the desert lakes of Ounianga, Chad (17). Triggered by these information an exploratory study was conceptualized with the aim to investigate whether trematode parasites such as *Schistosoma* spp occur in two settlements at the lakes of Ounianga, Ennedi Ouest province, in Northern Chad. The study was covering three aspects, namely epidemiology, malacology and the population's health priorities, their access to health care and treatment.

METHODS

Study site and study population

The study was carried out in January 2019 around the lakes and the two settlements of Ounianga Kebir and Ounianga Serir, Ennedi Ouest province in Northern Chad (Figure 1).

<<Figure 1 near here>><<**Fig 1. A map showing the lakes and the settlements of Ounianga Kebir and Ounianga Serir in Northern Chad.>>**

The official population estimates according to the latest national population census in Chad for Ounianga Kebir counts around 9000 people and for Ounianga Serir about 1000 people (RGPH2, 2009). In both communities, the primary schools were operational, yet not the secondary schools. The only functional health centre of the Ounianga district is located in Ounianga Kebir and its catchment population is estimated to include 30,000 people. Ounianga Serir has no functional health centre; the population has set up a health post to provide basic health services to the community members.

Epidemiological survey

The resident population of Ounianga Kebir and Ounianga Serir, older than 5 years of age, were eligible for participation. Sample size was calculated using Epi Info 7.1.3.3 (CDC). Parameters used were “population survey” with two-sided confidence intervals of 95%, an expected frequency of 50% and a population size of 10000, resulting in a sample size of 370. Proportional to the total population estimates, the targeted sample size repartition was 330 people in Ounianga Kebir and 40 people in Ounianga Serir. At household level and at the primary schools, individuals were randomly selected by applying the spatial sampling method from the Expanded Programme of Immunization (EPI) of the World Health Organization as previously published (18). After obtaining oral consent from each selected individual, or in case of children from their caretakers, they were asked to produce a urine sample. A mobile field laboratory was set up at the health centre, and health post, respectively. The urine samples were analysed for haematuria by reagent strip testing (Hemastix; Siemens Healthcare Diagnostics GmbH; Eschborn, Germany) and classified as negative, light and severe haematuria as outlined by the testing handbook. Subsequently, 10 ml samples were subjected to urine filtration, followed by microscopic screening of the filter content for the presence of *S. haematobium* eggs. A point-of-care circulating cathodic antigen (POC-CCA) urine cassette test (Rapid Medical Diagnostics; Pretoria, South Africa) was performed to screen for S. mansoni infections.

Qualitative survey

In both communities, one focus group discussion (FGDs) with men and one with women were organized. Additionally, one FGD was organized with the staff of the health centre in Ounianga Kebir. In Ounianga Serir, an in-depth interview (IDI) was carried out with the person responsible for the health post. The topics covered by the interview guides were disease priorities and priority health issues, perceptions and health seeking behaviour. FGDs and IDI were assisted by an interpreter who translated the conversation from Arabic to French, allowing the study team to take notes. Digital recordings of the FGDs and IDI were transcribed and translated into French, integrating the notes taken during the FGDs or IDI.

Malacological survey

Individual community members and school-aged children were asked to guide the team to human-water contact sites. At each site, GPS coordinates and the water parameters temperature (°C), pH, conductivity (µs/cm) and dissolved oxygen (mg/l) were recorded, using a portable multimeter (Hach®, HQ40D, Loveland, USA)). For turbidity, a trubidimeter was used (Formazin Nephelometric Units [FNU]; Hach®, 2100P Iso). The snail sampling was performed adhering to standard protocols. In short, for 15 minutes, all aquatic snails were collected by one person using a scoop or forceps to detach them from aquatic and subaquatic plants (19). Subsequently, the snails were placed on wet cotton in petri dishes, and transferred to the field laboratory. Snails were identified to the genus or, if possible to species level on site. At midday, each collected snail identified as intermediate host species was placed in water for three hours to induce cercarial shedding. The snail size (in mm) and weight (in mg) was measured using a calibre and balance, respectively. Thereafter, all snail specimens were conserved in 70% ethanol, and shipped to the National History Museum, London (NHM) for molecular analysis.

Molecular snail species and infections status confirmation

The snail samples selected for the molecular analyses represented individuals from each collection site. All specimens were stored in 70% ethanol in the field. On arrival at the NHM, the snail species identification was confirmed based on morphological characters and samples re-spirited (absolute ethanol) for incorporation into the Schistosomiasis Collection at the Natural History Museum (SCAN) (20). Photographic images were taken of the snail shells prior to DNA extraction. Specimens were placed in TE buffer (10mM Tris, 0.1mM EDTA) pH 7.4 for one hour in order to remove any remaining alcohol from within the tissue, which might interfere with subsequent extraction steps. Total genomic DNA was isolated from head/foot tissue using the DNeasy Blood and Tissue kit (Qiagen, UK) according to manufacturer's instructions. DNA was eluted into 200µl sterile water.

Amplification of *Cox1* fragments of snail DNA

A polymerase chain reaction (PCR) amplification of a partial cytochrome oxidase 1 (*Cox1*) sequence was performed using primers LCO1490 (5′GGTCAACAAATCATAAAG ATATTGG3′ forward) and HCO2198 (5′TAAACTTCAGGGTGACCAAAAAATCA3′ reverse) (21). PCR investigations and sequencing conditions were chosen as previously outlined (22, 23).

Checking of sequence data

The electropherograms produced were checked and *Cox1* sequences edited using Geneious, version 11.0.5 (http://www.geneious.com (24)). Sequences were compared to database entries by performing BLAST searches via the National Center for Biotechnology Information against GenBank and EMBL sequence databases; and aligned with reference material [3,4] using Geneious version 11.0.5.

Sequencing of *Schistosoma spp.* eggs in urine

Positive urine samples from Ouinanga Kebir were combined into 7 different pools of 8-12ml respectively one pooled sample of 12ml for the villages of Ouinanga Serir. Samples were shipped to the diagnostic center of the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland for further processing. There, each pool was centrifuged at 3000g for 10 minutes. Exactly 500µl of the pellet was re-suspended and transferred to a 2ml tube containing garnet beads. After addition of 1 ml PBS, the sample was centrifuged 1min at 13000g and the supernatant was discarded. The pellet with the garnet beads was frozen 30min at -80°C and further processed as described by Barda and colleagues (25, 26). Samples were first tested by simplex generic *Schistosoma spp.* 28S real-time PCR amplifying *S. mansoni*, *S. haematobium*, *S. intercalatum*, *S. bovis* (27) and additionally *S. japonicum* because of modifications added to the second reverse primer of the assay (**table 1**). The reaction mix contained 1x TaqMan GenExpression MasterMix (ThermoFisher Scientific, Basel, Switzerland), 800nmoles of forward primer, 400nmoles of each reverse primer and 200nmoles of probe. The samples were subsequently tested by a duplex real-time PCR for the presence of a specific *S. mansoni* TRE region and of *S. haematobium* dra1 sequence (27, 28). Each

reaction mix contained 1x TaqMan GenExpression MasterMix (ThermoFisher Scientific, Basel, Switzerland), 800nmoles of each primer, 200nmoles each probe (**table1**). The thermoprofile of all assays on the QuantStudio5 (ThermoFisher) consisted of 2min at 50°C, 10min at 95°C followed by 45 cycles of 15s at 95°C and 1min at 58°C. The specificity of all assays was previously tested on a variety of DNA from stool and blood samples including: *Ascaris lumbricoides, Blastocystis hominis, Cryptosporidium spp., Dientamoeba fragilis, Encephalitozoon spp., Endolimax nana, Entamoeba coli, E. dispar, E. histolytica, E. moshkovskii, E. polecki, Enterocytozoon bieneusi, Giardia lamblia, Hymenolepis nana, Iodamoeba bütschlii, Sarcocystis spp., Taenia spp., Strongyloides stercoralis, Trichuris trichiura, Plasmodium falciparum, P. vivax, P. malariae, P. ovale, Trypanosoma cruzi, T. brucei, Leishmania spp.* and was found to be 100% specific. Analytical limit of detection (LOD) was tested by a plasmid dilution row ranging from 10^{7} to 10^{-1} plasmids/µl containing an insert with the sequence of the *Schistosoma* real-time PCR product, and was found to be at 10 plasmids/µl for all assays. On each real-time PCR plate and for each target we included negative and positive low-copy plasmid controls.

Subsequently, all samples were tested by classic PCR of the COX gene of *S. haematobium* and *S. bovis* as modified from Boon and co-workers (Table 1) (29). The reaction mix contained 1x HotStarTaq Plus Master Mix (Qiagen, Hilden, Germany), 800nmoles of each Primer, 5µl DNA in a total reaction volume of 50µl. The thermoprofile consisted of 5min at 94°C followed by 40 cycles of 40s at 94°C, 40s at 58°C and 1min at 72°C and a final step of 10min at 72°C. After visualization on a 2% Agarose-Gel, the positive sample of the *S. bovis*-COX PCR was sent for Sanger sequencing with the primers of amplification at Microsynth AG (Baldach, Switzerland). The Sequence was then compared to database entries by performing BLAST searches via the National Center for Biotechnology Information. The sequence is accessible in GenBank under the number: MW937895. A table listing primers and probes is accessible in the supplementary materials.

Statistical analysis

Descriptive statistics of epidemiological and malacological data was performed using STATA version 16.0 (STATA Corp Inc., TX, USA) and ArcGIS (Version 10.7.1.; ESRI Inc. ArcMap™ 10.7, Redlands, CA, USA). Qualitative data analysis included full review of all transcripts, followed by a descriptive and explorative thematic analysis.

RESULTS

Epidemiological survey

In both study sites, urinary schistosomiasis was highly prevalent. Indeed, 35.3% (95% CI 28.7% – 41.8%) of the tested participants were *S. haematobium* egg positive in Ounianga Kebir and 54.9% (95% CI 40.8% – 69.0%) in Ounianga Serir, resulting in an overall prevalence of 39.2% (95% CI 33.2% – 45.1%) (Table 1).

Tab 1. *Prevalence of* S. haematobium *and* S. mansoni *infection, haematuria and infection intensities in the study population, Ounianga Serir and Ounianga Kebir, Chad, 2019.*

	Ounianga Kebir		**Ounianga Serir**		**Total**
	Male	**Female**	**Male**	**Female**	
Total number of participants	65	142	21	30	258
Participants age <18 years	47 (72%)	65 (46%)	14 (67%)	11 (37%)	137 (53%)
***S. haematobium* infection (egg positive)**					
Total no. positive	27 (41.5%)	46 (32.4%)	11 (52.4%)	17 (56.7%)	101 (39.2%)
Age <18 years*	24 (51.1%)	24 (36.9%)	9 (64.3%)	8 (72.7%)	65 (47.5%)
Heavy *S. haematobium* infection					
Total no. positive	14 (51.9%)	21 (45.7%)	8 (72.7%)	9 (52.9%)	52 (51.5%)
Age <18 years*	14 (58.3%)	14 (58.3%)	8 (88.9%)	7 (87.5%)	43 (66.2%)
POC-CCA test results					
Total no. positive	14 (21.9%)	17 (13.0%)	1 (4.8%)	5 (17.9%)	37 (15.2%)
Age <18 years*	14 (30.4%)	10 (16.7%)	1 (7.1%)	3 (27.3%)	28 (21.4%)

**(%) from all participants <18 year*

The *S. haematobium* prevalence was highest among children and adolescents below 18 years in both villages (Table 1). In Ounianga Kebir, more boys than girls were infected (51.1% *versus* 36.9%), whereas in Ounianga Seker girls had a higher prevalence (72.7%).

<<Figure 2 near here>>

<<Fig 2. Map showing the prevalence and snail abundance for Ounianga Kebir and Serir. The prevalence among participants is displayed by neighbourhood. For each water site sampled, the abundance of the intermediate host snail *Bulinus truncatus* is indicated.>>

Mapping of the schistosomiasis prevalence by place of living (neighbourhood) shows a slightly higher prevalence for those neighbourhoods closer to a water sites where the aquatic intermediate host snail *Bulinus truncates* was present (Fig 2, Ounianga Kebir: Yiggybeshi, Ounianga Serir: Roy). Regarding the POC-CCA testing for *S. mansoni*, 15.2% of the urine samples showed a positive test results.

More than half of all participants harboured a heavy *S. haematobium* infection (51.5%; heavy infection: >50 eggs/10 ml urine) and the burden was higher in children, whereof two third were heavily infected (Table 1) (30). Overall, 10.2% of all egg-negative, 69.4% of all light and 92.3% of all heavily infected participants had severe haematuria (Fig 3), with no big differences between age and gender.

<<Figure 3 near here>>

<<Fig 3. Schistosomiasis infection intensity and haematuria stratified by sex, age group and place of living.>>

Qualitative survey

During FGDs in both study sites, abdominal issues and blood in urine were the most frequently mentioned health problems among adults and also in children. Health staff mentioned that the majority of patients seeking care at the centre for any cause additionally suffers from abdominal issues. Among children, diarrhoeal diseases, respiratory infections and scorpion stings were reported as major health issues. Adults also suffered from eye problems, headache and joint pain. Fertility issues are another major concern and women reported difficulties getting pregnant again after they had their second or third child.

A major constraint for people in both sites is the difficult access to health facilities. Accessible facilities are usually underequipped; rarely have drugs available and the personnel had only basic training. To obtain appropriate care and treatment, people needed to travel long distances within Chad (to Faya, Abeche and N'Djamena) or abroad to Libya or Sudan.

Another common theme was the lack of safe drinking water as pumps are rare and open wells are commonly used as water sources. Perceived water quality is low due to salty taste and visible contamination.

The population was well aware of parasitic diseases, yet had limited knowledge on risk factors and transmission. Blood in urine was linked to parasitic infections, low quality of drinking water, water contact at the nearby lakes, or kidney issues. *Kadi* and *Kouli* are two local names for parasite infections linked to abdominal pain. *Kadi* describes an intestinal worm infection causing symptoms like intestinal spasms and flatulence, increased appetite with the tendency of weight loss As a traditional treatment, infected people are given natron or an extract of the roots of a plant called *Boa* to initiate diarrhoea, causing a worm with a red 'mouth' to leave the body via the excrements. The symptoms described for *Kouli* correlate with symptoms of the parasite *Enterobius vermicularis* such as persistent itching in the perianal area and sleep disturbances. The traditional treatment administered to *Kouli* patients are eating butter or drinking an extract of a medical plant called *Chi*.

Ouco is the local term to describe the health condition related to blood in urine combined with pain while urinating and reduced male erectile function. In traditional medicine, the urine of the animal called *Nii* (Fennec Fox, *Vulpes zerda*) is believed to have a curative effect.

The reported level of satisfaction with access to medical treatment for the above mentioned health issues is mixed. Important challenges mentioned included stock outs of medicines, lack of diagnostic means and non-effectiveness of the medical treatment received. Especially the female FGD participants expressed a need for health education and sensitization among the population.

Malacological survey

297 Among a total pf 17 different collection sites, 8 harboured fresh water snails (Tab 2, Fig 2).
298 Highest snail numbers were collected from the two intermediate host snail species *Limnea*
299 *natalensis* (n=42) and *Bulinus truncatus* (n=38), and two species of no medical importance,
300 *Gyralus sp.* and *Polypylis* sp. (n=42). Particularly high numbers of any snail species were
301 collected at two sites, namely Yoa 2 (n=35) in Ounianga Kebir and Agouta (n=34) in Ounianga
302 Kebir. Among all snails, only one *B. truncatus* was shedding cercariae (from Yoa 2). Upon
303 testing, they were recognized to not represent *S. haematobium* cercariae, and consequently
304 were not further studied. The average shell height of all *L. natalensis* specimen was 10.6mm
305 (95% confidence interval, 9.08mm - 12.11mm), 6.66 mm (95% CI, 5.97mm - 7.34mm) for *B.*
306 *truncates*, whereas all *Gyralus sp.* were juveniles with an average shell height of 1 mm or
307 below.
308 Across all sites where snails were found, the average water temperatures was 18.9°C
309 (standard deviation ±3.3), the average oxygen content was 4.8 mg/l (± 2.1) and a turbidity of
310 3.0 FNU (± 1.4). The sites without snails were characterized by a wide range of measured
311 water parameters, i.e. temperature of 22.1°C (range: 14.1-28.2), oxygen 5.9 mg/l (range: 1.1-
312 15.0) and turbidity 267.0 FNU (range: 1.0 - >1000.0). Snails obviously preferred the pH range
313 between 7.0 and 8.8 compared the sites without snails with a pH varying between 7.0 and
314 10.5. Inconclusive results were found for the conductivity comparing sites with and without
315 snails with a range of 6.0 to 1941.0 µs/cm and 2.8 to >2500.0 µs/cm, respectively.
316
317 Table 3. Snail abundance and water parameters for each sampling site

Sampling sites with snails present								
Site*		Snail species	No. of snails found	Temperature [°C]	Conductivity [µs/cm]	pH	Oxygen [mg/l]	Turbidity [FNU]
Ounianga Kebir								
Yoa (Girki)	1*	*Gyralus sp.*	1	17.5	1054.0	6.8	2.7	1.3
	2*	*B. truncatus*	28	14.5	1046.0	6.9	4.1	2.5
		L. natalensis	6					
Yoa (source 2)	3*	*L. natalensis*	19	22.7	1941.0	7.1	3.2	1.9
	4*	*L. natalensis*	7	22.8	2.56	7.0	3.0	3.7
Ounianga Serir								
Agouta		*Gyralus sp.*	27	14.5	8.0	8.3	4.9	3.5
		L. natalensis	7					
		V. nilotica	√					

Djara	*B. truncatus*	4	19.7	6.0	8.5	6.6	2.4
	G. ounaiangaensis	√					
	V. nilotica	√					
Boku	*B. truncatus*	6	20.8	1524.0	8.8	8.8	5.9
	L. natalensis	3					
	G. ounaiangaensis	√					
Bedrim	*G. ounaiangaensis*	√	18.7	7.3	8.4	5.2	3.0
Sampling sites without snails							
Ounianga Kebir							
Yoa 5 (hot source)			28.4	2.8	7.0	2.9	4.9
Yoa 6 (hot source)			27.7	2107.0	7.4	3.2	16.3
Yoa 7 (lake)			14.4	290.0	10.3	1.1	158.0
Uma red			27.5	>2000.0	10.1	14.2	85.8
Uma blue			18.6	>2000.0	10.5	15.0	118.0
Uma (hot spring)			28.2	4.0	9.1	3.6	14.9
Forodone			19.1	31.1	9.8	2.0	>1000.0
Ounianga Serir							
Telli			21.3	13.7	10.7	2.7	4.5
Edem			14.1	3.5	8.7	8.3	1.0

*All sampling sites are shown in Figure 2

Sequencing of *Schistosoma spp.* eggs in urine

All eight urine pools were positive in the generic *Schistosoma* spp. 28S real-time assay, in the *S. haematobium* dra1 real-time assay and in the *S. haematobium* COX1 PCR consistent with the presence of *S. haematobium* eggs in all pools. No pool was positive for *S. mansoni* TRE real-time PCR. One pool from the village of Ouinanga Serir was positive for *S. bovis* COX1. This result indicates the possibility of the presence of *S. haematobium* X *bovis* hybrids as observed in previous studies in West Africa (29, 31).

DISCUSSION

This exploratory study was the very first time a medical research team focused on the Sahara oasis of Ounianga, Ennedi Ouest province in Chad. We were able to show for the first time the high prevalence of *S. haematobium* in the population of both villages Ounianga Kebir and Serir. Living specimens of *B. truncatus* were found at both sites, whereas the previous findings were fossils dating back to the early Holocene (17). These findings suggest the possibility of ongoing local schistosomiasis transmission in this desert oasis environment.

The larger of the two villages, Ounianga Kebir had an overall lower schistosomiasis prevalence compared to the smaller village of Ounianga Serir (35% versus 55%). In the different neighbourhoods of Ounianga Kebir the prevalence varied and ranged from 21% to 42% (Fig 2). This may be partly explained by the proximity to the rare freshwater sites that are used for washing cloth, bathing and swimming. For example, Lake Yoa with its cold temperature and high salinity is fed by numerous freshwater springs. These provide habitats for the intermediate host snails, and the neighbourhood with the highest prevalence was the one closest to a freshwater spring. The quarters with lower prevalence were closer to freshwater sources including the two hot springs (Yoa 5 and 6). Here, the high water temperature might explain the absence of snails (32). The two adjacent sampling sites Yoa 3 and 4 are cold and only used for irrigating the surrounding gardens or for watering livestock. Interestingly, at these two sites snails of the species *Limnea natalesis* were found, the intermediate host of the liver fluke *Fasciola* spp. In Ounianga Serir, there are no hot springs and the two freshwater lakes (Djara and Boku) are used for all water-related activities. In both, *B. truncatus* were present and the lakes' close proximity to the quarter Roy may explain the high urinary schistosomiasis prevalence.

About half of the adult participants and two thirds of the children with a positive test suffered from heavy *S. haematobium* infections. Our data show that the infection intensity is associated with the severity of haematuria, pointing towards chronic schistosomiasis caused by long-term exposure and recurrent reinfection. Hence, the major health problems reported by the local population, namely abdominal issues and blood in urine, may well be due to schistosomiasis, and are likely the consequences of the lacking access to diagnostics and treatment options and the absence of any preventive intervention, as it has also been reported from other remote areas in Chad (33).

The study was set-up as an exploratory study with the aim to reveal the presence of the *Schistosoma* spp. lifecycle in the desert. Its scope is therefore limited and leaves several factors unaddressed at this stage. For example, the men's main activities involve working in soda extraction sites, trading using traditional caravans, and raising livestock through mobile

pastoralism. Hence, during the visit of the study team, the majority of men aged 16 to 60 years were absent resulting in an over-representation of women in the study population (ratio 1:2). Regarding the *S. mansoni* diagnostics that showed a positive POC-CCA result for 15.2% of all urine samples, we cannot conclude with certainty that *S. mansoni* is present in the study population as no stool samples were collected and hence, no parasitological proof of *S. mansoni* infection is available. Of note, according to the tests handbook, also a heavy infection with *S. haematobium* can lead to a positive test result (34). It is also significant that no intermediate host snails of the genus *Biomphalaria* were found. However, the exploratory study was conducted in January while snail abundance is highly seasonal (35).

Conclusion

This exploratory study presents the first modern evidence of urinary schistosomiasis among the population of these oasis villages. There is clearly a need for further studies to fully understand the current epidemiological situation. However, apart from further studies the main problems are already evident; namely the lack of health education, diagnostics and access to treatment. With a combined approach, including sensitization, mass drug administration, and morbidity management the control or even elimination of urinary schistosomiasis in this population might be possible.

Declarations

Ethics approval and consent to participate

The study received approval from the ethics committee Northwest and Central Switzerland (reference no. BASEC Nr Req-2018-0120) and the 'Comité National de Bioéthique du Tchad' (CNBT) in N'Djamena, Chad (reference no. 134/PR/MESRI/SG/CNBT/2018). Research authorization was granted by the Chadian Ministry of Health and its 'Direction de la Lutte contre la Maladie et de la Promotion de la Santé' (reference no. 007/PR/MSP/DG/DLMPS/2018).

Upon arrival in the study villages, an assembly was organized with the community representatives to discuss the study objectives and procedures. The traditional leaders, together with the local authorities, discussed the study and decided about concrete participation. Once a collective decision had been reached, written informed consent was obtained from the community representatives. In line with high illiteracy rates among the general population, individual participants consented orally. These consent procedures had received approval by the respective ethics committees. Those participants with a positive test result from either filtration or POC-CCA testing were invited to the health centre / health post and were administered praziquantel in the adequate dose (40 mg/kg) by the study nurse.

Consent for publication

All authors approved submission for publication of this manuscript.

Availability of data and materials

Data will be available on request by email to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HG conceived and designed the study protocol with input from PS; AAB, HG, RO and WM implemented the research in Chad; AAB, HG, MA, RO and WM carried out the field work and the parasitological examinations, together with a medical team; HG and WM sampled the snails, and FA and RC performed the genetic sequencing and analysis for species confirmation. RW and SP performed the genetic sequencing and analysis of the *Schistosoma*

samples. AAB, FA, HG, RO and WM analysed and interpreted the epidemiological data; HG, RO and WM drafted the manuscript; all authors critically revised the manuscript for intellectual content and approved the final manuscript. HG is the guarantor of the paper.

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538 SUPPLEMENTARY MATERIAL

539 **Table 1. Primers and probes**

Parasite	Name	Sequence 3'-5'	Origin
Generic *Schistosoma spp.*	Schisto28S_F	GTGGAGTTGAACTGCAAGC	Modified from Cnops et al. 2012
	Schisto28S_R1	CCATAGCAGACAGGCAGC	
	Schisto28S_R2	GCTCAACAWTAATAGTCAAACCTG	
	Schisto28S_P	FAM-ACTGACAAGCAGACCCTCACACC-BHQl	
S. mansoni TRE	Sman_F	CCACGCTCTCGCAAATAATCTA	Modified from Wichmann et al. 2013
	Sman_R	AAATCGTTGTATCTCCGAAACCA	
	Sman_P	YYE-ACAAACATCATAAAAATCCGTCCA-MGB-Q5	
S. haematobium dra1	Shae_F	GATCTCACCTATCAGACGAAAC	Identical to Cnops et al. 2013
	Shae_R	TCACAACGATACGACCAAC	
	Shae_P	FAM-TGTTGGTGGAAGTGCCTGTTTCGCAA-BHQ1	
S. haematobium COX	SH_COX_F	TTTTTTGGTCATCCAGAGGTGTAT	Modified from Boon et al. 2018
	SH_COX_R	TAATAATCAATGACCCTGCAATAA	
S. bovis COX	SB_COX_F	TTTTTTGGGCATCCGGAGGTGTAT	
	SB_COX_R	CACAGGATCAGACAAACGAGTACC	

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