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# Urinary Schistosomiasis in Remote Communities of Kouh-Est and Grande Sido in Southern Chad

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## Abstract

Schistosomiasis, the world's second-largest parasitic endemic, is caused by trematodes of the genus *Schistosoma*. This study aimed to determine the prevalence of urinary schistosomiasis in Kouh-Est and Grande Sido in southern Chad. A survey was conducted in 18 villages, including sedentary and nomadic populations, involving 783 participants. PCR and DNA sequencing were performed to identify and characterize Schistosoma species. DNA for diagnostic confirmation, epidemiological studies, and understanding genetic diversity. The overall prevalence of *Schistosoma haematobium* was 31.54%. In Kouh-Est, the prevalence was 60,72% and in Grande Sido, it was 39.27% This prevalence was higher in the age groups 11-15 years (19.83%) and 16-20 years (10.52%). The difference between distribution rates and age groups was statistically significant ($P<0.05$). The prevalence was higher among uneducated individuals (50%), with primary and secondary education levels at 20%, and religious education at 10%. Schistosomiasis is present in Kouh-Est and Grande Sido. Mass drug administration, health education, and community mobilization are crucial strategies to significantly reduce the prevalence of schistosomiasis in these communities.

**Keyword:** Prevalence; *Schistosoma haematobium;* Kouh-Est; Grande Sido; Chad.

## 1. Introduction

Schistosomiasis is one of the most widely spread parasitic helminthic infections that affect humans. It is an occupational risk encountered in rural areas of developing countries, where potable water is scarce. The disease is characterized by the presence of blood in the urine and sometimes by pain during or after urination. In 2017, the WHO estimated that 250 million people were affected by the disease in 52 countries worldwide, with 800.000 deaths recorded annually [1]. Some studies have shown that nearly 800 million people worldwide are exposed to the disease [2]. Schistosomiasis has been associated with growth failure, malnutrition, cognitive impairment, and reduced work capacity. It is transmitted by freshwater mollusks, which serve as intermediate hosts [3,4]. Many studies confirm that the distribution of schistosomiasis is strongly linked to the physical environment, such as hydro-agricultural developments, which create a context favorable to the development of this disease [5,6]. The highest prevalence rates are recorded in tropical and subtropical regions, particularly among communities living along rivers [7-8]. Humans contract the disease when they come into contact with infected water bodies while carrying out necessary daily activities such as farming, fishing, laundry, bathing, and swimming [5]. Schistosomiasis is prevalent in many parts of rural Africa, Latin America, South America, and Asia, leading to severe morbidity and negative health outcomes in many endemic communities.

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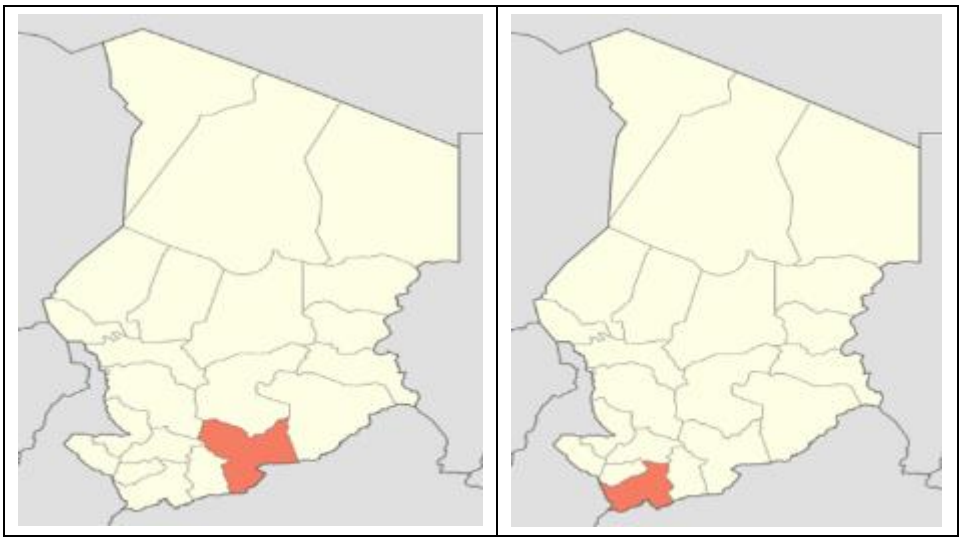
The disease manifests in forms ranging from intestinal to urinary or co-infections, depending on the parasite species and the body part where the eggs are deposited. The severity of the disease is often related to the patient's age, initial immune status, and immune response to infection, with pathologies such as hydronephrosis, hematuria, or liver damage. These socioeconomic activities and symptoms are not uncommon among the inhabitants of Grande Sido and Kouh-Est, in southern Chad, which are characterized by numerous rivers, ponds, irrigated farming, and borrow pits. Grande Sido and Kouh-Est have diverse freshwater environments that offer numerous favorable habitats for aquatic snails, which serve as intermediate hosts. A few studies have been carried out in Chad; for example, urinary schistosomiasis prevalence rates of 26.4%, 24.9%, 39.2%, and 11.5% have been found in N'Djamena and Abéché in the Sahelian zone, in the Torrock and Rong populations in the Sahelo-Sudanian zone, and in the population living around the Ounianga lakes in the Sahara [9-10]. In addition, a recent study reported a 55% hematuria rate due to *Schistosoma haematobium* infection in school-age children in the Salamat region of the Sudanian zone [11]. The aim of this study was to determine the prevalence of urinary schistosomiasis in Kouh-Est and Grande Sido in the southern part of Chad. To achieve this objective PCR and DNA sequencing were used performed. The sequencing of *Schistosoma* DNA is a critical step in identifying and characterizing genetic material of the parasite, essential for diagnostic confirmation, epidemiological studies, and understanding genetic diversity.

## 2. Materials and method

### 2.1. **Study area and population**

This cross-sectional and prospective study was conducted in Kouh-Est, located at 8°12.375'N, 17°08.063'E in the Logone-Oriental province, and Grande Sido, located at 8°24.807'N, 18°46.139'E in the Moyen-Chari Province (Figure 1). The annual rainfall in this area ranges from 600 mm to 1000 mm [12]. There are two seasons: the rainy season from May to October and the dry season from November to April [13]. The population of Kouh-Est was approximately 38,600, and that of Grande Sido around 14,500 inhabitants distributed across 45 settlements [14]. Households were selected as described in Ibrahim *et al*., 2021[14], and Ibrahim *et al*., 2025 [15].



| **Grande Sido (a)** | **Kouh-Est (b)** |
|---|---|

**Figure 1** Study areas (National Centre for research support CNAR, 2017).

### 2.2. Inclusion criteria

According to the inclusion criteria, participants were required to consent to participate in the study, be between 0 and 90 years of age, and reside in a household selected in the area of Kouh-East or Grande Sido.

### 2.3. Exclusion Criteria

Subjects who refused to participate in the study or were not from the area of Kouh-East and Grande Sido were excluded from the study.

### 2.4. Ethics statement

Before data collection, ethical clearance approving this study protocol was obtained from the National Bioethics Committee of Chad under N°585/PR/PM/MESRI/SEESRI/SG/2016. Sampling survey authorizations were also obtained from the Ministry of Public Health.

### 2.5. Urine Collection

Urine collection cups, labeled with participants' ID numbers, were distributed to each household for morning urine collection (10 to 30 mL). Parents collected urine samples from younger children.

### 2.6. DNA extraction, molecular amplification and identification, subcloning and sequencing of amplicons

DNA was extracted from urine samples using the QiAamp DNA Mini Kit (Qiagen®) according to the manufacturer's instructions. After incubating the samples with AL buffer and proteinase K, ethanol was added, and the mixture was purified using a purification column. The DNA was then eluted into AE buffer.

Photometric quantification of the extracted DNA at a wavelength of 260 nm was performed using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany) (Ibrahim *et al.,* 2021) [14], yielding concentrations ranging from 50 to 300 ng/µL. The DNA extracts were stored at -20°C.

PCR was performed to amplify a specific gene Dra1 repeat sequence of *Schistosoma haematobium*. A 25 µL master mix was prepared, containing 1 µL of extracted DNA, 2 µM of specific primers [30]. (forward: GATCTCACCTATCAGACGAAAC and reverse: TCACAACGATACGACCAAC), 200 µM dNTPs, 2.5 units of DreamTaq polymerase, 4 µL of the appropriate buffer, and $ddH_2O$.

The PCR conditions were as follows: initial denaturation at 98°C for 4 minutes, followed by 30 cycles of denaturation at 98°C for 10 seconds, annealing at 54°C for 10 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes.

A 12 µL aliquot of each PCR product was separated on 1.5% agarose gels prepared with TBE (Tris-Borate-EDTA) or TAE (Tris-Acetate-EDTA) buffer. The gels were stained with Stain-G (SERVA, Heidelberg, Germany) and electrophoresed for 1 hour at 100V. 5 µL molecular marker (GeneRuler DNA ladder, Thermo Scientific) was loaded alongside the samples to estimate the sizes of the expected amplicon fragments. Selected PCR products of amplified gene fragments were excised from the agarose gel and purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. DNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany).

The purified DNA was subcloned into a linearized pJET 1.2/blunt plasmid using the CloneJET PCR Cloning Kit (Thermo Fisher Scientific) following the manufacturer's instructions. Single clones were screened by colony PCR using inner ITS1 primers. Positive clones were picked and cultured overnight at 37°C with shaking (220 rpm) in 5 mL of LB medium containing 100 µg/mL ampicillin. The following day, plasmids were purified using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific). The purified DNA, combined with one of the inner primers, was sent to a commercial provider (Microsynth SeqLab, Göttingen, Germany) for Sanger sequencing [16].

### 2.7. Sequences read and phylogenetic tree construction

Sequences were read, analysed and aligned using Geneious Pro 5.5.9. Alignments of sequences of *S. haematobium* were done with Gap open penalty 15 and Gap extension penalty 5. The sequences were aligned against the GenBank database using nucleotide BLAST from NCBI. MEGAX software was used to investigate the phylogenetic relationship of *S. haematobium* based on their Dra1 sequences. The DNA sequences were imported from Geneious and aligned with the MUSCLE algorithm method together with the reference sequences retrieved from the GenBank database. The evolutionary history was inferred using the Neighbour-Joining method. The evolutionary distances were computed with the Maximum Composite Likelihood method.

### 2.8. Data analysis

All data obtained during the study were entered into Microsoft Excel 2010 and analyzed using SPSS version 25.0. The Chi-square test was used to compare variables such as age, sex, and other parameters, with the p-value threshold set at 0.05.

## 3. Results

### 3.1. Human survey data

During our survey, 783 participants were randomly selected. Males predominantly headed households, with 55.0% (95% CI: 48.5 - 61.9%) in monogamous and 20.8% (95% CI: 15.3% - 26.7%) in polygamous settings. In contrast, female-headed households were less common (9.9%; 95% CI: 5.9% - 13.9%) and primarily comprised widows (35.0%; 95% CI: 15.0 - 55.0%; n=7), divorced women (35.0%; 95% CI: 15.0 - 55.0%; n=7), or single women (30.0%; 95% CI: 10.0 - 50.0%; n=6). The overall participant gender distribution was nearly equal, with 50.3% (95% CI: 47.1 - 53.6%) male and 49.7% (95% CI: 46.4 - 52.9%) female.

Sedentary populations were more highly represented than nomadic populations. The nomadic groups, primarily pastoralists, migrated to these areas in search of grass and crop residues for their livestock, staying for over six months in some cases. Additionally, the survey included a military camp located in the Grande Sido area.

Eight villages/settlements were visited in the Kouh-East focus, while ten were visited in the Grande Sido, including one military camp located near one of the visited villages (Table 1). The lowest number of participants in a village was recorded in Guirkyon Village (n=9), and the highest was in Kousserie Village (n=90).

**Table 1** Distribution of participants according to ages and villages.

| Zone | Village | 0–20 ans n (%) | 21–40 ans n (%) | 41–90 ans n (%) | Total n (%) |
|---|---|---|---|---|---|
| Kouh-Est | Bembaïtada | 26 (41.3) | 21 (33.3) | 16 (25.4) | 63 (14.7) |
| | Konael | 21 (43.8) | 16 (33.3) | 11 (22.9) | 48 (11.2) |
| | Kobiteye | 29 (50.9) | 9 (15.8) | 19 (33.3) | 57 (13.3) |
| | Dedaye I | 18 (40.9) | 13 (29.5) | 13 (29.5) | 44 (10.2) |
| | Ferrick Sandana Le-lou | 10 (35.7) | 12 (42.9) | 6 (21.4) | 28 (6.5) |
| | Kousserie | 60 (66.7) | 20 (22.2) | 10 (11.1) | 90 (20.9) |
| | Palkoyo | 18 (28.1) | 32 (50.0) | 14 (21.9) | 64 (11.8) |
| | Berayan | 20 (55.6) | 6 (16.7) | 10 (27.8) | 36 (11.6) |
| Total Kouh-Est | — | 202 (47.0) | 129 (30.0) | 99 (23.0) | 430(54.9) |
| Grand Sido | Ngakorio | 34 (41.5) | 27 (32.9) | 21 (25.6) | 82 (23.2) |
| | Ferrick Hanno | 20 (36.4) | 17 (30.9) | 18 (32.7) | 55 (15.6) |
| | Kobdogué | 18 (32.1) | 21 (37.5) | 17 (30.4) | 56 (15.9) |
| | Ngon-Molo | 11 (44.0) | 8 (32.0) | 6 (24.0) | 25 (7.1) |
| | Beguiyon | 9 (36.0) | 10 (40.0) | 6 (24.0) | 25 (7.1) |
| | Baguirgué : Guir-ba | 7 (26.9) | 16 (61.5) | 3 (11.5) | 26 (7.4) |
| | Guirkyon / C. militaire | 15 (62.5) | 4 (16.7) | 5 (20.8) | 24 (6.8) |
| | Baguirgué / C. militaire | 10 (25.6) | 21 (53.8) | 8 (20.5) | 39 (11.0) |
| | Aldjazira | 4 (33.3) | 5 (41.7) | 3 (25.0) | 12 (3.4) |
| | Guirkyon | 3 (33.3) | 4 (44.4) | 2 (22.2) | 9 (2.6) |
| Total Grand Sido | — | 151(42.8) | 133(37.7) | 69(19.5) | 353(45.1) |
| Total général | — | 353 (45.1) | 262 (33.5) | 168 (21.4) | 783 (100) |

**Legend**: Participants were stratified into three age groups (0–20, 21–40, and 41–90 years) across villages. The majority were aged 0–20 years in both Kouh-Est (47.0%) and Grand Sido (42.8%).

The samples analyzed in this study came from the two departments Grand Sido and Kouh-Est; the contribution of each department in the supply of urine samples. Grand Sido department contributed 430 urines, while Kouh-Est department provided 353 urines, 31,54 % and 68,45% of samples respectively.

**Table 2** Distribution according to location (Kouh-Est and Grande Sido)

| **Province** | **Positif n (%)** | **Négatif n (%)** | **Total n (%)** |
|---|---|---|---|
| Kouh-Est | 150 (60.72) | 280 (39.28) | 430 (54.92) |
| Grand Sido | 97 (39.27) | 256 (60.73) | 353 (45.08) |
| **Total** | 247 (31.54) | 536 (68.45) | 783 (100) |

Distribution of *Schistosoma spp.* screening results by province (Kouh-Est and Grand Sido). Data are presented as counts and percentages (%). The "Positive" column indicates the number of infected individuals, while the "Negative" column corresponds to non-infected individuals. The total combines both provinces (n = 783).

Of the 783 individuals who participated in this study, 247 (31.54%) were infected with *S. haematobium*, including 97 (25.73%) females and 150 (36.95%) males.

### 3.2. Distribution according to sex groups

**Table 3** Sex distribution.

| | **Total n (%)** | **Positive n (%)** | **P-value** |
|---|---|---|---|
| Male | 377 (48.14) | 97 (25.73) | 0.001 |
| Female | 406 (51.85) | 150 (36.95) | |
| Total | 783 (100) | 247 (31.54) | |

Distribution of *Schistosoma spp.* infection according to sex. Data are presented as counts and percentages (%). The chi-square test indicated a statistically significant difference between males and females (p = 0.001).

### 3.3. Distribution according to age groups

The age groups 11-15 years and 16-20 years had the highest infection rates, with prevalence of 20.64% and 10.52%, respectively.

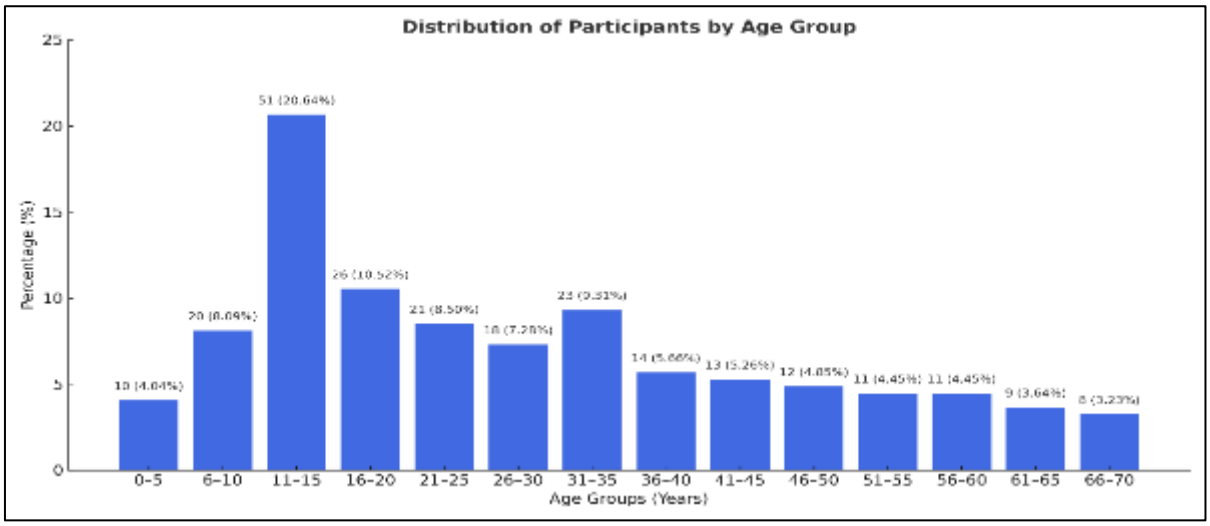



**Figure 2** Distribution by age group

**Legend** The figure shows the percentage of study participants distributed across different age groups, with the highest proportion in the 11–15 years group and the lowest in the 66–70 years group.

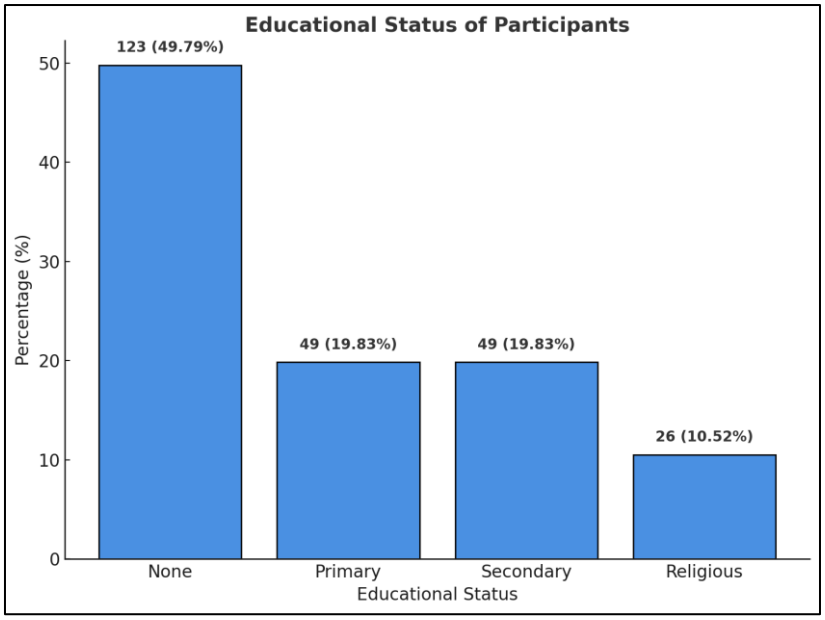



**Figure 3** Distribution by educational status.

According to Figure 3, our results showed that 49.79% of infestations occurred among uneducated participants, 19.83% among those with primary and secondary education, and 10.52% among those from religious schools.

**Legend:** Nearly half (49.79%) had no formal education, 19.83% had reached primary level, 19.83% secondary level, and 10.52% reported religious education.

**Molecular identification and subcloning result**

The results of experiments using *S. haematobium* genomic DNA isolated from the urine of negative and positive specimens as a template showed that the 121 bp amplicon and the higher-order forms of the Dra1 repeat were easily visualized on an agarose gel in Figure 3 (negative specimens: lane 2, 3, 6, 7, 8, 9, 12, 13, 14 and 15; and positive specimens: lane 10 and 11). This result was consistent for all positive specimens (10 samples: 1,27%) of this study. It was deemed reliable and indicative of *S. haematobium* infection.

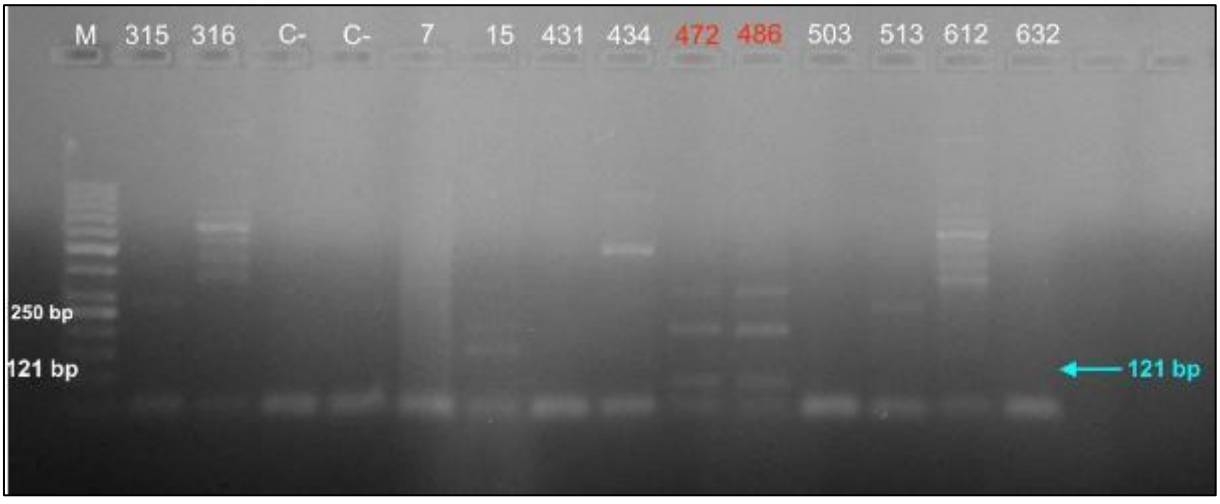



**Figure 4** Detection of 121 bp of DraI repeat sequence of *Schistosoma haematobium*

Detection of 121 bp of DraI repeat sequence of *Schistosoma haematobium* employing Sh primers [17]. M = gene ruler 50 bp size markers; C- = negative control; lanes (315, 316, 7, 15, 431, 434, 503, 513, 612, 632) = negative samples from Chad; lanes (472, 486) = positive samples from Chad.

To ensure the purity and quality of the sequence for downstream analysis, the PCR product was subcloned into a linearized pJET 1.2/blunt plasmid using the CloneJET PCR Cloning Kit (Thermo Fisher Scientific). The ligated vector was transformed into *E. coli* XL1-Blue. Colony PCR was used to screen positive clones, confirming successful insertion of the 121 bp DNA fragment. Plasmids from positive clones were further verified by restriction enzyme digestion, ensuring the presence of the target DNA.

The subcloned product was sequenced using Sanger sequencing, which provided high-quality reads. Alignment of the sequence using the BLAST tool showed a 98% match with the DraI gene of *S. haematobium*, a key marker used for species identification.

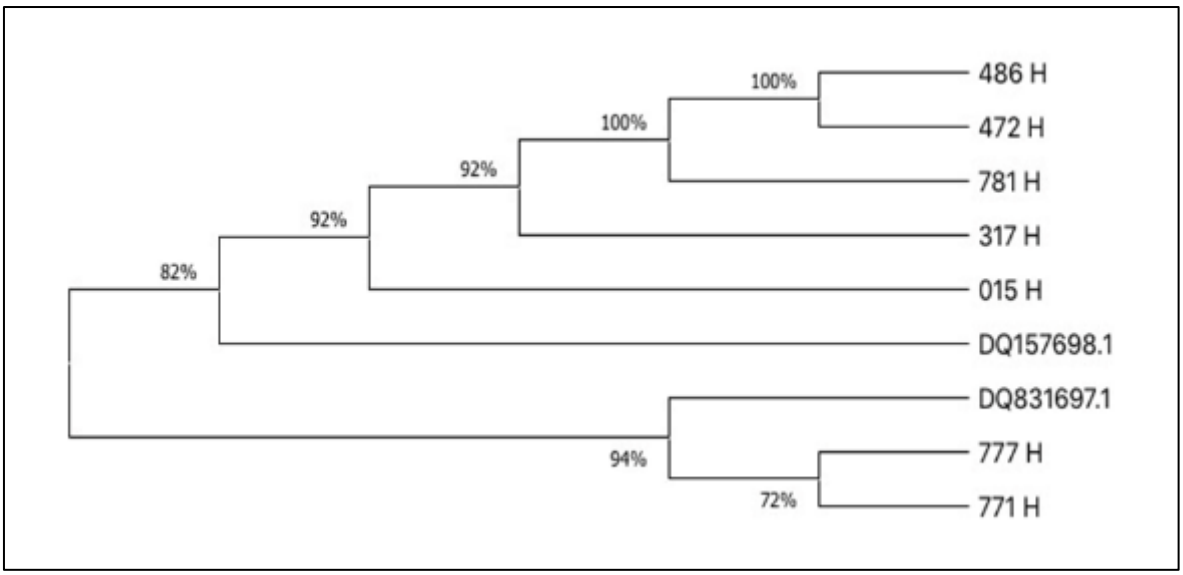



**Figure 5** Evolutionary analysis by Maximum Likelihood method of 9 nucleotide sequences

### 3.4. Evolutionary analysis by Maximum Likelihood method of 9 nucleotide sequences

(7 of the studied urine samples: 486 H, 472 H, 781 H, 317 H, 015H, 777 H and 771 H; 2 references from NCBI: DQ157698.1 and DQ831697.1).

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [18]. The tree with the highest log likelihood (-595.64) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree [19]. This analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 138 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [20].

## 4. Discussion

*Schistosomiasis* is prevalent across Chad, notably in the Moyen-Chari and Logone Oriental provinces. The intricate and fluctuating transmission dynamics are influenced by intermediate host bionomics, human behavior, and environmental variables. This study aimed to determine the prevalence of urinary schistosomiasis in southern Chadian communities, specifically Kouh-Est (Logone Oriental) and Grande Sido (Moyen-Chari), encompassing diverse remote rural and nomadic villages with demographic variations as detailed in Ibrahim *et al.*, 2025[31],

The samples analyzed in this study came from the two departments Grand Sido and Kouh-Est; the contribution of each department in the supply of urine samples. Grand Sido department contributed 430 urines, while Kouh-Est department provided 353 urines, i.e. 55.30% and 40.22% of samples respectively, similar to that of Bello *et al.,* 2003 [16],

Of the 783 individuals who participated, the overall infection rate was 31.54%. This finding is lower than the prevalence rates reported in Sudan (54.2%) [21], Nigeria (47%) [22] and the 55% observed in Chad's Salamat province [23]. These geographical variations in prevalence likely reflect differences in environmental factors, intermediate host distribution, and community exposure patterns.

Our results revealed a higher infection rate in males (36.95%) compared to females (25.73%), consistent with studies by Diepreye *et al.,* (2021) [17] and Akinneye *et al.,* (2018) [18], which attributed this to increased cultural and occupational exposure of males to infested water. Similarly, Tarek *et al.,* (2020) [24] in Egypt reported higher infection rates and egg counts in males across all age groups.

The age groups 11–15 and 16–20 years showed the highest prevalence in our study, at 19.83% and 10.52%, respectively (Figure 3). These findings mirror those of Dawaki *et al.,* (2016) [25], who reported high rates among children ≤10 and adolescents aged 11–20 in Kano State, Nigeria. This age-related trend aligns with several studies [25], suggesting that schistosomiasis prevalence peaks during adolescence and declines thereafter—likely due to increased water contact through swimming, bathing, and play. Similar trends were observed in Nigeria, Mauritania, and Niger, where infection rates rose in children aged 6–13 and declined after age 14 [25].

In terms of education level, 49.79% of infected individuals in our study were uneducated, while 19.83% had primary or secondary education, and 10.52% attended religious schools. This supports findings by Abdel-Wahab *et al.,* (2000) [26] in Sudan and Mahamat *et al.,* (2023) [27] in Abéché, Chad, who also reported higher infection rates among less-educated populations. The increased prevalence among uneducated participants may be due to lower awareness of transmission risks and common practices like bathing in contaminated water. Additionally, proximity to transmission sites, competent snail hosts, and environmental factors contribute to this distribution.

On a molecular level, the successful amplification, subcloning, and sequencing of *Schistosoma* DNA in our study not only confirm the parasite's identity but also enrich the genetic database of *Schistosoma* species in endemic regions. This sequence provides valuable insight into local genetic diversity, transmission dynamics, drug resistance, and potential vaccine targets. The small molecular sample size (n = 10) is a limitation of this study. Although selected to capture variation across localities and clinical profiles, these samples may not represent the full microscopy-positive population. Consequently, the phylogenetic results should be viewed as exploratory. Larger molecular studies are warranted to validate and extend these findings, which could ultimately support the development of more accurate diagnostic tools for *Schistosoma* in both clinical and field settings.

## 5. Conclusion

This study confirms that schistosomiasis remains prevalent in the communities of Kouh-Est and Grande Sido in southern Chad. To effectively combat the disease in these areas, public health authorities should expand screening efforts to include other community members and ensure timely treatment of infected individuals. Beyond mass drug administration, school- and community-based health education programs promoting proper hygiene and sanitation practices are essential to significantly reduce transmission and morbidity associated with schistosomiasis.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that they have no conflicts of interest.

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*Statement of informed consent*

Yes, informed consent was obtained from all individual participants included in the study.

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