Detection and identification of cyclops in well's water, Jabal Awliya locality, Khartoum, Sudan 2021 [version 1; peer review: awaiting peer review]

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Abstract

Background: Dracunculus medinensis or "Guinea-worm" is the parasitic worm that causes Dracunculiasis disease. The Cyclops (Meso and Micro Cyclops), which is only spread through drinking from water sources in endemic areas, carries this worm. This study aimed to detect and identify Cyclops in wells water, Jabal Awliya locality Khartoum state, Sudan 2021

Methods: This is a cross sectional study held in Jabal Awliya locality which is located in Khartoum state Sudan from 2021-2022. The locality is one of the seven localities in the state which is considered as the main gate for the refugees from South Sudan, Chad and Ethiopia where Dracunculus medinensis has not been eradicated yet. The population is 274,321.

Results: A total of 264 samples were collected, 132 from well's water and 132 from post wells water sources. Well's water contamination with cyclops was found to be 2.4% while post well's contamination was found to be 8.9%. The species reported by microscopy are: Afrocyclops, Mesocyclops and Microcyclops. Significant statistical difference between well's and post well's contamination was found (p-value 0.02).

Conclusions: In conclusion, it is apparent that well's water as well as dam water are contaminated with copepods (Afro-, Meso- and Microcyclops). Hence Residents in the research regions are at hazard for Dracunculus infection whenever larvae make their way to the water sources since mesocyclops and microcyclops are recognized.
Guinea worm vectors. Since no apparent water treatment system has been found in the areas under study, it is crucial that water sources in this area be treated before consumption in order to reduce the risk of illness. Awareness by the worm as well as the disease should be raised in order to ensure that the larvae from the hosts will not be carried to the wells.

**Keywords**
Cyclops, dracunculus Medinensis, wells, Sudan, copepods, mesocyclops, microcyclops
Introduction

Dracunculus medinensis or “Guinea-worm” is a parasitic worm that causes Dracunculiasis disease. It is one of the large tissue parasites that affect human beings. The adult female is between 600 and 800 mm long and 2 mm in diameter. There are around 3 million embryos inside. The cycle is completed by the parasite migrating through the victim's subcutaneous tissues after ingestion of the infected stage, which causes excruciating pain, especially in the joints. Most of the time, the worm eventually pops out of the feet. It results in fever, nausea, and vomiting along with an extremely painful oedema, blister, and ulcer.

Dracunculus and dracunculiasis issue has been discussed in the seminal review by Ralph Muller via serial publication since 1971. In order to promote a campaign to eradicate dracunculiasis, the Centers for Disease Control and Prevention and the Carter Center formed a partnership of organizations. In 1986, endemic dracunculiasis was reported to exist in 18 of 20 nations, including Sudan (Ruiz-Tiben and Hopkins 2006). Elimination of 98% of the estimated 3.5 million cases of dracunculiasis (Guinea worm disease) that existed less than 20 years ago has been achieved. The majority of the remaining patients are in southern Sudan, and the global eradication campaign cannot be finished until the Sudanese civil war is over, despite the fact that seven of the 20 countries that were endemic for the disease have already eradicated the disease (Hopkins and Withers 2002).

The primary source of water supply in Africa is still surface water, which is obtained through small-scale dams, hafirs, ponds, and wells, many of which are man-made. Given that they are dispersed widely and primarily affect isolated rural communities, their impact on human health has gone unnoticed. According to World Health Organization studies, the disaster is that water can spread more than 80 dangerous diseases. Dracunculiasis is one of the illnesses and is brought on by the worm Dracunculus medinensis. The Cyclops (Meso and Micro Cyclops), which are only transmitted through drinking from water sources in endemic areas, carries this worm. It is well recognized that small-scale dams and lagoons contribute to the prevalence of dracunculiasis (Ilegbodu et al. 1987, Cairncross and Tayeh 1988). Theoretically, tiny dams put a lot more people at risk of disease transmission than huge dams, however this has been contested (Jewsbury and Imevbore 1988). On the other hand, not many studies have discussed the transmission of the disease through the wells.

As the result of southern Sudan war many of south Sudanese travel to north Sudan, especially the Jabal Awliya locality, that became a refugee camp during the Second Sudanese Civil War, housing more than 100,000 inhabitants. It became a gate way for South Sudanese refugees. The presence of a source of infection -which is the refugees in the camps or another hosts like dogs- and the vector- the Cyclops - as well as the environment -surface water- makes the mission of total eradication of Dracunculus Medinensis in Sudan very difficult.

No many studies in the literature regarding the water contamination with the Cyclops or the larvae of Dracunculus specially in Sudan have been found. This study aimed to detect and identify Cyclops in Well’s water, Jabal Awliya locality, Khartoum state, Sudan 2021.

Methods

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of the University of Bahri - Faculty of Medicine. Administrative approval from the authority of water, Jabal Al Awliya locality, Khartoum was obtained. Data were aligned anonymously and held with a high level of confidentiality.

This was a cross sectional study held in the Jabal Awliya locality which is located in Khartoum state, Sudan from 2021-2022. The locality is one of the seven localities in the state which is considered as the main gate for the refugees from South Sudan, Chad and Ethiopia where Dracunculus medinensis has not been eradicated yet. The population is 274,321.

The main water source in Jabal Awliya is wells water. The design of the well is illustrated in (Figure 1). The water from wells passes into treatment stations then to the tanks. From tanks water passes to the houses to be available for the household use in form of tap water.

Collection of samples

The total number of samples was 264. Samples were collected from wells. Post well water was sampled also. For each area supplied by a certain well a single house was chosen using non-random sampling (convenient) due to unavailability of sufficient data regarding the houses lists. Tap water was sampled in three clean tubes cautiously.

All the samples were transferred to the laboratory of The University of Khartoum, Faculty of health. Firstly, a light microscope (Olympus BX compound microscope) manufactured by New York Microscope Company
(OLBX40F-PATH-40/R) was used to detect the cyclops. Then Cyclopidae were isolated by passing the water samples through special-mesh sieves. All specimens were preserved in ethanol (96%).

**Dissection of Cyclops for morphological identification**

The dissection and identification were done in the laboratory of The University of Khartoum, Faculty of health. Only female specimens were selected for identification; these were carefully transferred with fine dissecting pins from the petri-dish to a drop of water-free glycerine manufactured by Merck KGaA, Damstadt, Germany (catalogue No. 104057) on a glass slide. For identification of cyclopoid copepods, the fine structures of the antennary basipodite segment, the 4th pair of legs, 5th pair of legs and maxillulary palp were of paramount importance. The specimens were placed on the dorsal side

**Figure 1. The design of wells.**

**Figure 2. The tools used for preparation of the water samples.**
with a drop of glycerine; the abdomen was separated from the rest of the body with one dissecting pin. The tools used for preparation of the samples are illustrated in Figure 2.

Each specimen was cut between the thoracic somites three and four, and then the P4 was separated. The abdomen was placed on the dorsal side with the ventral side facing upwards, in order that the 5th pair of legs, the genital somite with the receptaculum seminis and the furcal rami with setae, could be observed. The first antennules were also separated. The 4th thoracic segment was separated from the abdomen of the specimen and placed with the caudal site facing upwards in order that the 4th pair of legs and the uniting lamella can be seen (Idris and Mohamed 2015). Examination of species was done by an Olympus BX 50 compound microscope.

The identification of the genus Mesocyclops was mainly done by using “The Guides of the Identification of the Microinvertebrates of the Continental waters of the World” (Holyńska et al. 2003).

Results

A total of 264 samples were collected, 132 from well’s water and 132 from post wells water sources. Well’s water contamination with cyclops was found to be 2.4%, while post well’s contamination was found to be 8.9%. The species reported by microscopy were: Afrocyclops, Mesocyclops and Microcyclops. Significant statistical difference between well’s and post well’s contamination was found (p-value 0.02) (Table 1).

Another sample that was collected from Jabal Awliya dam showed contamination with mesocyclops. Pictures for the microscopic identification of the mesocyclops are illustrated in Figure 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th>Well’s samples</th>
<th>Post well’s samples</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4 (2.4%)</td>
<td>12 (8.9%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Negative</td>
<td>128 (97.6%)</td>
<td>120 (91.1%)</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>132</td>
<td>132</td>
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Figure 3. Mesocyclop seen under microscope.
Discussion

The Guinea worm eradication program has been firstly established in 1992. Since that time great efforts are being done in order to eradicate Dracunculiasis as well as prevent the reemergence of the disease. Total eradication has been achieved in North Sudan since 2013. The South Sudanese health minister declared that Guinea worm transmission had ended within the country in March 2018 at the Carter Center. The disease has a 12-month life cycle, and the most recent case was documented 15 months ago (Carter, 2018). Unfortunately reemergence has occurred. The number of human cases has been in the double digits for the previous eight years (54 in 2019 and 27 human cases in 2020). These human cases have been documented in six nations: Angola (1 case), Chad (12 cases), Ethiopia (11 cases), Mali (1 case), South Sudan (1 case), and Cameroon (1 case). They were probably imported from Chad. Many factors raise the risk of importing new cases from the previously mentioned countries. First of all being surrounded with the majority of the endemic countries. Besides that, people of those countries suffer from poor socio-economic status as well as an unstable political situation. As a result of these factors, many people travel to north Sudan, particularly Jabal Awliya territory, which has become a displaced person camp amid the Moment Sudanese Respectful War, lodging more than 100,000 tenants. It got to be a gate way for refugees suffers poor socio-economy, lack of basic health services, high levels of illiteracy leading to malpractices (The March of the Green Flag 1995). What makes travel of refugees easier is that the boarders of Sudan are open and not well secured. The presence of the vector (cyclops) and the sources of infection (refugees and dogs) within the same area creates a risk of reemergence of Guinea worm infection in Sudan.

One of the crucial precautions that must be taken to stop the Guinea worm transmission cycle from being completed is water safety. Since 2013 the surveillance regarding presence of the vector, as well as the infective agent, has been halted. This study is a part of the surveillance that should be kept specially in this locality aiming to assess the safety of water sources regarding Guinea worm transmission.

The detection and identification of the copepods - one of the important hosts that transmit dracunculus medinensis - in drinking water is one of the corner stones of the eradication process. In the present study, water from different levels (before and after treatment) as well as Jabal Awliya dam has been assessed microscopically.

The microscopy results showed that well’s water contamination with copepods was 2.4%, while post well’s contamination was 8.9%. The identified genera are Mesocyclops, Microcyclops and Afrocyclops. On the other hand, samples from the Jabal Awliya dam showed contamination with mesocyclops. Both Mesocyclops and Microcyclops act as vectors for Guinea worm as reported by Johnson (1990). The presence of those types of copepods in drinking water creates a suitable environment for Guinea worm. In other words, whenever worms find their way to water sources, the life cycle will be completed and hence there will be a reemergence of the disease. Copepods are very sensitive for water treatment.
with simple methods including chlorination, so the detection of such crustaceans in drinking water is an indicator for inefficient treatment. A noteworthy measurable distinction between well’s and post well’s contamination was found (p-value 0.02). The nonappearance of clear water treatment system can be a conceivable clarification for the high rate of contamination of the post-well water. A high species diversity of Cyclopidae was discovered in a subsequent study carried out in Nigeria in 2020 by Yijun Ni et al. pointing to investigate the species diversity and distribution of copepods in freshwater habitats using a mitochondrial cytochrome c oxidase subunit I marker. From the Cyclopidae, 15 populations contained five Tropocyclops species, five Mesocyclops species, and two Thermocyclops species. Numerous conceivable reasons can be considered as clarification of this results difference. First of all, the difference in the water sources from which samples were collected. On the other hand, the variability of water treatment systems used in different countries could be a possible reason. Moreover, the diversity in temperature, humidity, PH and collection seasons can be a coherent clarification for contamination contrast. Lastly, the utilization of molecular techniques by Yijun Ni et al. for distinguishing proof of copepods can be considered as a conceivable cause for this contrast (Yijun Ni et al., 2020).

The high percentage of post-well contamination indicates a high chance of infection, since the presence of the vector raises the transmission potential of any infectious disease. In case of contamination of water with the fecal material of infected animals, larvae will find their way to the vector (Cyclops). Completion of the worm life cycle will occur whenever people get access to this water. The mission of Guinea worm infection elimination will be unachievable in the presence of high transmission potential. On the other hand, eradication of dracunculiasis as a global goal will be impeded.

A total of 816 water samples from wells, streams, boreholes, and rainfall in the research areas were gathered for a different study conducted by Simon-Oke, Afolabi, and Obimakinde in Nigeria. About 10% of the samples were reportedly contaminated with Dracunculus medinensis and Enterobius vermicularis. This percentage concerns the larvae stage of Dracunculus medinensis which is the infectious stage to the human being (Simon-Oke et al., 2020). In comparison with the current study, a higher chance of illness spread was found in Nigeria at the time of the mentioned study. The contrast between the two studies can be clarified in a way similar to the study by Yijun Ni et al.

**Conclusion**

In conclusion, it is apparent that well’s water as well as dam water are contaminated with copepods (Afro-, Meso- and Microcyclops). Hence Residents in the research regions are at hazard for Dracunculus infection whenever larvae make their way to the water sources since mesocyclops and microcyclops are recognized Guinea worm vectors.

**Recommendations**

Since no apparent water treatment system has been found in the area under study, it is crucial that water sources in this area be treated before consumption in order to reduce the risk of illness. In addition, since some wells are not well structured and not protected, dogs - one of the animal hosts - have easy access to them. Moreover, rain can drift directly to wells carrying fecal materials and contaminated soil. So these wells should be restructured and covered carefully.

Awareness of the worm cycle as well as the disease should be raised in order to ensure that the larvae from the hosts will not be carried to water sources.

**Data availability**

**Underlying data**


This project contains the following underlying data:

- athar data English.xlsx (This is an excel sheet summarizing the results of microscopic identification of the water samples collected from different sources included in this study).
- Figure 3 mesocyclops under microscopy.tif (this is a picture of the mesocyclops positive water sample).
- Figure 4 mesocyclops under microscopy.tif (this is a picture of the mesocyclops positive water sample).

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0).
Acknowledgments

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References


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