



## Case Report

Human infection with an unknown species of *Dracunculus* in VietnamPham Ngoc Thach<sup>a</sup>, H. Rogier van Doorn<sup>b,c</sup>, Henry S. Bishop<sup>d</sup>, Mark S. Fox<sup>d</sup>, Sarah G.H. Sapp<sup>d</sup>, Vitaliano A. Cama<sup>d</sup>, Le Van Duyet<sup>a,\*</sup><sup>a</sup> National Hospital for Tropical Diseases 2, Hanoi, Vietnam<sup>b</sup> Oxford University Clinical Research Unit, Hanoi, Vietnam<sup>c</sup> Centre of Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK<sup>d</sup> Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

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## ABSTRACT

Guinea worm (GW) disease, caused by *Dracunculus medinensis*, is an almost eradicated waterborne zoonotic disease. The World Health Organization (WHO) currently lists GW as endemic in only five African countries. In July 2020, the Vietnamese public health surveillance system detected a hanging worm in a 23-year-old male patient, who did not report any travel to Africa or any country previously endemic for GW. The patient was hospitalized with symptoms of fatigue, anorexia, muscle aches, and abscesses, with worms hanging out of the skin in the lower limbs. The worms were retrieved from the lesions and microscopically examined in Vietnam, identifying structures compatible with *Dracunculus* spp. and L1-type larvae. A section of this parasite was sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, United States, for confirmatory diagnosis of GW. The adult worm had cuticle structures compatible with *Dracunculus* parasites, although the length of L1 larvae was about 339 µm, substantially shorter than *D. medinensis*. DNA sequence analysis of the 18S small subunit rRNA gene confirmed that this parasite was not GW, and determined that the sample belonged to a *Dracunculus* sp. not previously reported in GenBank that clustered with the animal-infective *Dracunculus insignis* and *Dracunculus lutrae*, located in a different clade than *D. medinensis*. This study highlights the importance of effective public health surveillance systems and the collaborative work of local public health authorities from Vietnam with the WHO and CDC in efforts to achieve the eradication of GW.

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## Introduction

The *Dracunculus* worms belong to the superfamily Dracunculoidae that commonly parasitize the tissue and serous cavities of mammals, fish, reptiles, amphibians, and birds. Among the species of *Dracunculus*, the most widely known and studied is *Dracunculus medinensis*, also known as the Guinea worm (GW), which was historically endemic in regions of Africa, the Middle East, and South Asia. Recent data from the World Health Organization (WHO) showed that the number of worldwide infections with GW

had decreased from 3.5 million people in 1986 to 28 in 2018 (World Health Organization, 2020a). By February 16, 2018, there were 198 countries recognized as free of GW (World Health Organization, 2020a). In 2019, only 54 human cases were reported worldwide: one in Angola, 48 in Chad, four in South Sudan, and one in Cameroon (Hopkins et al., 2020). However, infections of *D. medinensis* in other mammals, mainly dogs but also felids and baboons, along with new insights into transmission pathways involving transport and paratenic hosts like fish and amphibians, has raised awareness of the potential emergence of GW in novel areas (Hopkins et al., 2020; Molyneux and Sankara, 2017). The emergency was further highlighted by the detection of *D. medinensis* infections in Angola, a country located in an African region not previously endemic for GW (Hopkins et al., 2017). Here, we report the clinical manifestations, therapies, and conclusive

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E-mail address: [duyetibt@gmail.com](mailto:duyetibt@gmail.com) (L.V. Duyet).<http://dx.doi.org/10.1016/j.ijid.2021.02.018>1201-9712/© 2021 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

diagnosis of a hanging worm removed from a patient in Vietnam with no known travel history. This study highlights the importance of effective public health surveillance systems and the collaborative work of local public health authorities from Vietnam with the WHO and the US Centers for Disease Control and Prevention (CDC) in efforts to achieve the eradication of GW.

## Methods

### Microscopy methods

A worm fragment of about 1 cm long, preserved in 95% ethanol, was submitted to the US CDC Parasite Reference Diagnostic Laboratory. The sample and fragments in the transport fluid were viewed under differential interference contrast with an Olympus BX51 compound microscope. Length and width measurements were taken using cellSens Imaging Software, standard version 2.2 (Olympus Life Sciences, Waltham, MA, USA). Comparative images and measurements were also captured for *D. medinensis* first-stage larvae originating from a human case in Chad.

### DNA isolation

Following microscopic examination of the sample, a section of 5 mm was used for DNA extraction. Briefly, DNA was extracted using the Qiagen Investigator Kit (Qiagen Sciences, Maryland, USA) as per the manufacturer's instructions for forensic samples, and DNA was eluted twice from the filter membranes to maximize the amount of DNA collected. The first and second eluates of DNA were separately amplified by PCR and sequenced.

### PCR amplification of the 18S ribosomal RNA gene

PCR was performed as described previously (Bimi et al., 2005) using a commercially available master mix (Platinum Green PCR Supermix; Invitrogen, Carlsbad, CA, USA). Briefly, each reaction had 25 µl of master mix, 20 µl H<sub>2</sub>O, 4 µl of each forward and reverse primer (Supplementary Material, Appendix 1), and 1 µl of DNA template. PCR amplification was performed with the following cycling parameters: an initial 15 min denaturation step at 95 °C, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing for 30 s (first cycle at 60 °C, decreasing by 2 °C every other cycle until reaching 54 °C for the final 39 cycles), and elongation for 90 s at 68 °C. This was followed by a final soaking cycle at 28 °C for 10 min. The PCR products were electrophoresed and visualized in 1.5% agarose gels. Positive samples would have an expected size of 1800 bp.

### Sanger sequencing

Nematode 18S PCR products were submitted for bidirectional Sanger sequencing at the Genomic Sequencing Lab of the Biotechnology Core Facility Branch, CDC using sequencing primers described previously (Qvarnstrom et al., 2007) (Supplementary Material, Appendix 1).

### Phylogenetic analysis

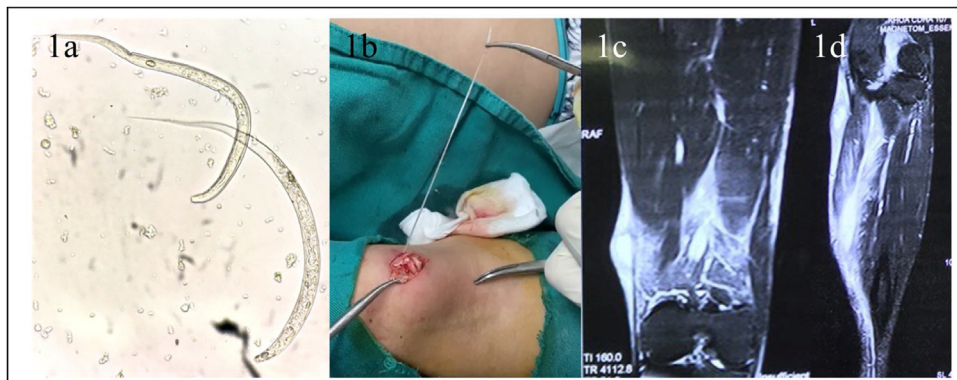
Ten sequence fragments each for the duplicate 18S PCR products were manually trimmed, assembled using ChromasPro v2.1.9 (Technelysium Pty. Ltd, South Brisbane, Australia), and subjected to quality control using the online NCBI BLAST tool (blast.ncbi.nlm.nih.gov). Multiple sequence alignments were made in Geneious v. 11.0.3 (Biomatters, San Diego, CA, USA) using the 1812 bp sequence of the 18S gene from this report, 56 representative sequences of *D. medinensis*, three sequences from non-medinensis *Dracunculus* species that infect animals, and eight nematode species. A phylogenetic neighbour-joining tree was generated with the Geneious Tree Builder Tool using the Tamura–Nei model and bootstrap set at 1000; *Ascaris suum* was set as the outgroup.

### Case presentation

The patient was a 23-year-old male from Yen Bai Province, with no travel history to endemic or formerly endemic parts of Africa/South Asia. For about a year before presentation of the hanging worms, the patient felt tired, ate poorly, had sore muscles in his leg, and had a papular rash on the calf and thigh area that developed into purulent abscesses. The patient had no other systemic or local symptoms. He had visited many commune, district, and provincial-level health facilities before presenting at the National Hospital for Tropical Diseases, but no diagnosis was made.

On May 12, 2020, the patient was seen at the National Hospital for Tropical Diseases, where besides clinical manifestations, doctors found several abscesses in the thigh, calf, and neck areas. The patient had marked eosinophilia (12.1%,  $1.8 \times 10^9/l$ ), but no other abnormal laboratory findings. Computed tomography of the heart, lungs, and brain did not find any abnormalities. Ultrasound of the legs showed several lesions scattered over the thighs and lower legs (Figure 1c, d).

The patient received thiabendazole to facilitate the manual removal of hanging worms, which was performed slowly until the worms were entirely removed from the lesions (Figure 1b). The patient took thiabendazole for 4 weeks and did not show additional parasites by the time treatment was completed. The abscesses were needle-evacuated, and visible adult worms were



**Figure 1.** (a) L1 larvae of *Dracunculus* collected from a femoral abscess. (b) *Dracunculus* worm extraction surgery on the patient's upper arm. (c), (d) Images of soft tissue lesions under the skin of the patient's thighs and lower legs.

observed from the contents, while nematode-like structures and larvae were further observed on microscopic examination (Figure 1a). Over the course of the patient's hospitalization, a total of five abscesses were drained and cleaned and a total of five adult worms measuring 30–60 cm were removed from the patient's arms and legs (Figure 1b).

## Diagnosis

A 1 cm section of one worm was submitted to the CDC for morphological and molecular evaluation.

By microscopy, the sample had morphometric characteristics of *Dracunculus* spp. Additionally, *Dracunculus* first-stage larvae were detected, of which 10 were measured: length was an average of 339  $\mu\text{m}$  (range 318–377  $\mu\text{m}$ ) and width was an average of 20  $\mu\text{m}$  (range 19–21  $\mu\text{m}$ ). This measurement excluded *D. medinensis* from the diagnosis (581–643  $\mu\text{m}$ ), as well as *Dracunculus insignis* (596–857  $\mu\text{m}$ ), *Dracunculus lutrae* (608–722  $\mu\text{m}$ ), and *Dracunculus globocephalus* (666–721  $\mu\text{m}$ ), based on a compilation of measurements from valid *Dracunculus* spp. (Cleveland et al., 2018). A comparison to-scale of larval size versus *D. medinensis* is shown in Figure 2. The observed range is consistent with various described reptilian *Dracunculus* species, although insufficient features of the adult worm were available to make a species-level diagnosis.

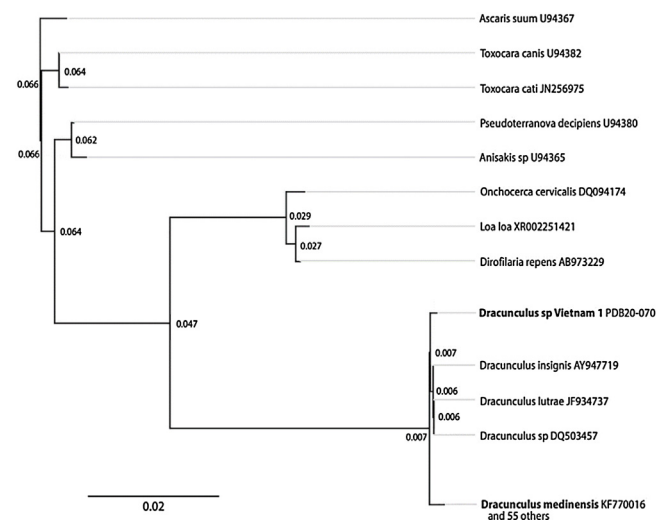
From the DNA analyses, the PCR reactions had positive amplification of the whole 18S gene and were sequenced independently. Individual and consensus sequences from the amplified products (fragment size approximately 1820 nucleotides) were analysed by BLAST. The results matched multiple species of the genus *Dracunculus*. The phylogenetic analysis located the sample sequences in a *Dracunculus* clade with *D. insignis* and *D. lutrae*, but different from *D. medinensis* (Figure 3).

## Discussion

In 1986, it was estimated there were 3.5 million cases of dracunculiasis in 20 endemic countries in Asia and Africa (World Health Organization, 2020a, b). The WHO in partnership with The Carter Center, a non-governmental, not-for-profit organization founded by former US President Jimmy Carter, then established the International Dracunculiasis Eradication Programme (Center). The number of GW cases has since been reduced by more than 99%, with only 22 cases in 2015. In 2018, a total of 198 countries were certified as GW-free by the WHO (World Health Organization, 2020b). In 2019, 54 cases were still reported from four countries in



**Figure 2.** A *Dracunculus* sp. larva from the case presented in this report (left) shown under differential interference contrast (DIC) to scale versus a *Dracunculus medinensis* L1 larva from Chad (right). Scale bars = 100  $\mu\text{m}$ .



**Figure 3.** Neighbour-joining tree based on 18S gene sequence data including *Dracunculus* sp. from Vietnam (bold), 56 sequences of *Dracunculus medinensis* (bold) reported in GenBank (node was collapsed) and from samples previously diagnosed by the US CDC, reference samples from non-*D. medinensis* *Dracunculus* spp., and eight human-infecting nematode species, using *Ascaris suum* as the outgroup for tree topology. Scale bar indicates substitutions per site.

Africa (South Sudan, Chad, Angola, and Cameroon) (World Health Organization, 2020b).

GW disease could become the second human disease in history, after smallpox, to be eradicated. It would be the first parasitic disease to be eradicated and the first disease to be eradicated without the use of a vaccine or medicine. A challenge to eradication has been the emergence of numerous GW infections in animals including domestic dogs. In 2019, the WHO revised the goal for eradication in humans and animals to 2030 (previous targets have been set at 1991, 2009, 2015, and 2020).

Therefore, it is critical to differentiate infections caused by *D. medinensis* from those caused by other *Dracunculus* species. One of the challenges for the identification and descriptions of *Dracunculus* spp. in natural definitive hosts, as well as humans, is the limited number of differentiating morphological features available in adult female worms, which are the hanging worms. Most females are microscopically indistinguishable among the species described. However, there is some inter-species variation in the size of L1 larvae, particularly between mammalian and reptilian-infecting *Dracunculus* spp. (Cleveland et al., 2018). DNA sequencing of the 18S gene is therefore very useful, to determine if a sample is *D. medinensis* or not. However, DNA sequences alone are limited for the species identification of non-medinensis *Dracunculus* spp. due to the very small number of sequences available for BLAST analyses.

In this case, the combined morphological and molecular analyses definitively excluded *D. medinensis* as the causative agent, pointing to a potential aberrant infection with an unknown zoonotic *Dracunculus* sp. The observed range of larval lengths was consistent with various described reptilian *Dracunculus* species and excluded many mammalian species.

Currently there are nine described species of *Dracunculus* in reptiles, with the majority in Eurasia and Africa, including *D. colubensis*, *D. alii*, *D. houdemeri*, *D. doi*, *D. dahomensis*, and *D. oesophageus*. *Dracunculus mulbus* is found in Oceania, and two species (*D. ophidensis* and *D. brasiliensis*) have been described in the Americas. Notably, *D. houdemeri* has been described in Vietnam from checkered keelback snakes, *Fowlea piscator* (Cleveland et al., 2018), and L1 measurements were consistent with the specimen

reported here. However, given the lack of additional morphological identifying features and the fact that no sequence data are available for most animal *Dracunculus* spp., particularly among reptiles, assigning a precise species to this diagnosis was not possible.

No cases of GW have ever been reported in mainland Southeast Asia. Probable cases of zoonotic transmission of non-*D. medinensis* species to people with no travel history to endemic areas have been documented sporadically, including reports from Japan, Korea, and Indonesia (Cairncross et al., 2002; Kobayashi et al., 1986). Although *D. houdemeri* has been documented in checkered keelback snakes, this is the first report of *Dracunculus* infecting humans in Vietnam. The patient lived in the northern highlands of Vietnam, where there is a tradition of eating raw foods including vegetables and fresh fish from local rivers. Given the lack of endemicity of *D. medinensis* in Vietnam and the very long pre-patent period, it is not possible to elucidate whether this infection was acquired through drinking water containing infected copepods or through fish carrying L3 larvae as transport or paratenic hosts, or any other potential route.

In conclusion, we describe an unusual case of *Dracunculus* sp. infection in a Vietnamese person. The patient was treated with a special medication regimen (thiabendazole) in combination with worm removal surgery. The natural hosts and route of exposure of this presumably zoonotic *Dracunculus* sp. have yet to be resolved and warrant further investigation and surveillance for similar cases in humans and animals in the region. This study highlights the importance of effective public health surveillance systems demonstrated in this collaborative work between public health authorities from Vietnam, the WHO, and the US CDC.

## Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

## Ethics approval and consent to participate

Not applicable.

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The research received no external funding.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ijid.2021.02.018>.

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