

# The First Human Case of *Thelazia callipaeda* Infection in Vietnam

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**Abstract:** A 26-year-old man residing in a village of Thai Nguyen Province, North Vietnam, visited the Thai Nguyen Provincial Hospital in July 2008. He felt a bulge-sticking pain in his left eye and extracted 5 small nematode worms by himself half a day before visiting the hospital. Two more worms were extracted from his left eye by a medical doctor, and they were morphologically observed and genetically analyzed on the mitochondrial cytochrome *c* oxidase 1 gene. The worms were 1 male and 1 female, and genetically identical with those of *Thelazia callipaeda*. By the present study, the presence of human *T. callipaeda* infection is first reported in Vietnam.

**Key words:** *Thelazia callipaeda*, oriental eyeworm, eye, human case, Vietnam

## INTRODUCTION

Zoonotic parasites are widespread in the world, especially in Asian countries, including Vietnam. *Thelazia callipaeda* Railliet and Henry, 1910 (Nematoda: Thelaziidae) is a nematode parasite in the genus *Thelazia* [1]. This nematode is zoonotic and parasitic in the eyes as implied by its name “oriental eyeworm” or “eyeworm”. It was reported for the first time from a dog in Pakistan in 1910, and later shown to be widespread in China, France, Germany, India, Indonesia, Italy, Japan, Korea, the Netherlands, Russia, Switzerland, Taiwan, Myanmar, and Thailand [2]. The final hosts include dogs and cats, but occasionally rabbits, monkeys, raccoons, dogs, foxes, wolves, and humans can also serve as the final host [3]. Intermediate hosts are insects, such as flies. It is relatively well recognized that drosophilid flies but not *Musca domestica* are the vector hosts for *T. callipaeda* [4-6]. Also in Japan, 3 species of the genus *Amiota* (Drosophilidae), namely, *Amiota okadai*, *A. magna*, and *A. nagatai*, have been identified [3].

The adult worm is parasitic in the conjunctival sac of a final host, and gives larvae continually by ovoviviparity. When a fly

licks the tear in the eye of a final host, including humans, the larvae enter the conjunctival sac, and become adults in 1 month after 2 molts [1,3]. Symptoms of *T. callipaeda* infection include conjunctivitis, excessive watering, visual impairment, and ulcers or scarring of the cornea [7]. In some cases, the only symptom is the presence of worms obscuring the host's vision as a floater [7].

With regard to the nematode infection in the human eyes in Vietnam, *Dirofilaria repens* was recently reported from the human conjunctiva [8]. However, *T. callipaeda* infection has never been reported in Vietnam. In this study, we report for the first time a case of human *T. callipaeda* infection in Vietnam which was verified by both morphology and molecular analysis using the mitochondrial cytochrome *c* oxidase 1 (*cox1*) gene.

## CASE RECORD

The patient was a 26-year old male residing in the Cao Phong village, Hop Tien commune, Dong Hy district, Thai Nguyen Province of mountainous North Vietnam. In July 2008, he felt a bulge-sticking pain in his left eye and extracted 5 small nematode worms by himself but he did not keep them. This symptom did not disappear after collection of worms himself. Then, half a day later, he visited the Thai Nguyen Provincial Hospital and a medical doctor collected 2 more worms. These worms were identified morphologically and also by a molecular method. The morphology of the worms was not so good, but they

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were thin, long, and cylindrical with milkish-white color. One was a male, 10 mm in length and 0.4 mm in width, with a curled tail end, and the other was a female, 15 mm in length and 0.5 mm in width, with a slender tail end. The female worm had a scalariform buccal cavity, a long muscular esophagus and a conical tail, and the vulva opening at the anterior portion of the esophago-intestinal junction. Comparison with the figure described as *T. callipaeda* in Miyazaki [3] showed that these worms were *T. callipaeda* (Nematoda: Thelaziidae).

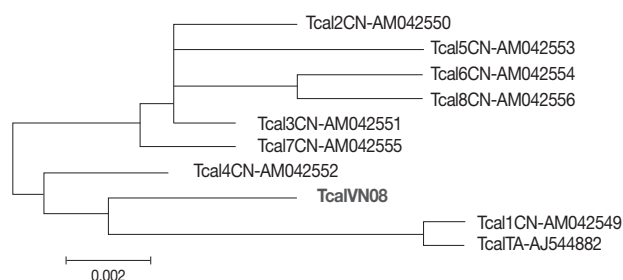
To support the morphological diagnosis, PCR analysis was performed on the *cox1* gene with the primer of THELF-THELR for the Vietnamese *Thelazia*. Total 627 nucleotide and 209 amino acid sequences of the *cox1* gene were compared with those of the previously known strains or isolates reported in GenBank (Table 1). There were 7 nucleotide differences in the Vietnamese *T. callipaeda*, but no changes in the amino acids at the changed places. The Vietnamese isolate had high homologies (98-99%) with 8 *T. callipaeda* isolates from China in GenBank (No. AM042549-AM042556) and 1 from Italy (No. AJ544882) [9]. However, the homology of our isolate with *Thelazia gulosa* (No. AJ544881) from Italy was low, 86% (Table 1). A phylogenetic analysis between the Vietnamese *T. callipaeda* and standard strains or isolates in the world showed that *T. callipaeda* from Vietnam and *T. callipaeda* from China and Italy is an identical group (Fig. 1).

## DISCUSSION

The first human case of thelaziasis was reported in China in 1917, and later from India, Thailand, Korea, Russia, and Japan [3]. In Japan, there were 30 cases until 1981 [3]. In Korea, 39 human cases were reported until 2011, and a total of 146 adult

worms were collected from the patients [10]. However, in Vietnam, this is the first time when *T. callipaeda* infection is reported from a human patient. The worms were parasitic in the conjunctival sac of an eye of a 26-year-old man. He felt a bulge-sticking pain in his left eye and no other symptoms. A total of 7 worms were extracted, but 5 worms collected by the patient himself were lost and only 2 were available. Although the worm morphology was not so good, they were identified as 1 male (with a curled tail end) and 1 female (with a straight, slender tail end), and could be identified as *T. callipaeda*. The flies of the family Drosophilidae, intermediate hosts for *T. callipaeda*, are very common in Vietnam. This zoonotic disease can be transmitted from animals to humans.

In order to support the morphological diagnosis, the nucleotide and amino acid sequences of the *cox1* gene of our Vietnamese worms were compared with those of 9 isolates of *T. callipaeda* reported in GenBank (8 from China and 1 from Ita-



**Fig. 1.** A phylogeny tree of *Thelazia callipaeda* from Vietnam and other parts of the world in GenBank using the *cox1* gene. TcalVN08: a sample of *T. callipaeda* from Vietnam; Tcal (1, 2, 3, 4, 5, 6, 7, 8) CN: samples from China (No: AM042549-AM042556); TcalITA: a sample from Italy (No: AJ544882). A phylogenetic analysis between the Vietnamese *T. callipaeda* and standard strains or isolates in the world showed that *T. callipaeda* from Vietnam and those from China and Italy is an identical group.

**Table 1.** The results of *cox1* gene analysis on the Vietnamese isolate of *Thelazia callipaeda* compared with other known isolates in GenBank

GenBank No.	Item	Detected point	% Comparison	Homology (%)
AM042552.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h4	1,125	100	99
AM042552.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h7	1,114	100	98
AM042552.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h3	1,114	100	98
AM042549.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h1	1,114	100	98
AJ5444882.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I	1,114	100	98
AM042550.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h2	1,109	100	98
AM042556.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h8	1,107	99	98
AM042554.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h6	1,107	99	98
AM042553.1	<i>Thelazia callipaeda</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I, haplotype h5	1,108	100	99
AJ544881.1	<i>Thelazia gulosa</i> mitochondrial partial CO1 gene for cytochrome oxidase subunit I	699	100	86

ly). The results showed that there were 7 places of nucleotide differences in our isolate but no changes in the amino acid sequence at the changed places. Thus, the amino acid homology between the Vietnamese isolate and 8 other isolates in the world was 100%, and only 1 isolate, number 4 (GenBank no. AM042552), revealed 99% homology with our isolate (difference in 1 amino acid, i.e., phenylalanine). Therefore, our worms were identified by morphology and molecular methods as *T. callipaeda*.

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