

Zoonotic Helminthes Parasitizing Human Eyes in Vietnam. Morphological and Molecular Identification of the Causal Agent

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Abstract

Zoonotic helminthiasis are widespread in Vietnam. Some of these cause ocular helminthiasis in humans. A total of 15 worms collected from human eyes, included 9 cases of dirofilariasis, 3 cases of thelaziasis, one case of toxocariasis, one case of angiostrongyliasis, and one case of gnathostomiasis. All worms were identified by species based on morphological and molecular methods. Five species of ocular helminths, including *Toxocara canis*, *Dirofilaria repens*, *Angiostrongylus cantonensis*, *Thelazia callipaeda*, and *Gnathostoma spinigerum* were detected in Vietnam.

Keywords: *Toxocara Canis*; *Dirofilaria Repens*; *Angiostrongylus Cantonensis*; *Thaeazia Callipaeda*; *Gnathostoma Spinigerum*

Introduction

Ocular helminths may cause blindness with severe socio-economic consequences to human communities [1]. Ocular helminthiasis infections are distributed worldwide and comprise nematodes such as *Onchocerca volvulus*, *Loa loa*, *Toxocara canis/Toxocara cati*, *Angiostrongylus cantonensis*, *Dirofilaria repens*, *Trichinella spiralis*, *Thelazia callipaeda*, *Baylisascaris procyonis*, *Wuchereria bancrofti/Brugia malayi*, and Cestodes including *T. solium cysticercus*, *Echinococcus granulosus*, and *Multiceps multiceps* larvae from different geographical areas [2]. Five of these related to this report are *Dirofilaria*, *Toxocara*, *Thelazia*, *Angiostrongylus*, and *Gnathostoma*. Dirofilariasis is prevalent worldwide and is an important zoonotic infection that has been reported in increasing numbers from Mediterranean countries such as Italy, France, Greece and Spain, and have also been reported in Croatia, India, Serbia, Denmark, Russia and Tunisia [3]. Thelaziasis has a geographical distribution in the Asia-Pacific region (China, India, Thailand, Indonesia, Japan and Korea) and Russia [4]. Toxocariasis is the most common in humans worldwide, particularly in Asia, Japan, Korea, Ireland and Alabama [2]. Angiostrongyliasis has been detected in Italy, China, Taiwan, Madagascar, Hawaii, South-East Asia and Japan [5]. A total of 73 cases of ocular gnathostomiasis were reported from 1939 to 2009 in 12 countries, including Japan, China, Vietnam, Philippines, Cambodia, Thailand, Malaysia, Myanmar, Bangladesh, India, Israel and Mexico [6]. Helminths at the adult and/or larval stages may infect human ocular tissues externally such as eyelids, conjunctiva sacs, subconjunctiva, and lachrymal glands, or the ocular globe such as the optical nerve, intravitreal retina, and the anterior and posterior chambers [4]. Most of these are zoonotic parasites. This report includes some cases of ocular helminthiasis in Vietnam.

Methods

A literature search was performed using international and national papers until 2018. To collect material for this study, a detailed search of the published database was performed using any term related to ophthalmology. The terms were ocular helminths in Vietnam and collected the worms for identification species by morphological and molecular method. That were including toxocariasis, dirofilariasis, angiostrongyliasis, thelaziasis and gnathostomiasis.

Results

The most important ocular diseases caused by helminths in Vietnam include toxocariasis, dirofilariasis, angiostrongyliasis, thelaziasis and gnathostomiasis.

Ocular diseases	No of cases	Sex/age*	Chief complaints	Size of worm
Toxocariasis	1	M/34 YO	Loss of vision	0.5 mm
Dirofilariasis	1	F/50 YO	Loss of vision	40 mm
	2	M/47 YO	Eye pain	80 mm
	3	F/27 YO	Loss of vision	100 mm
	4	M/49 YO	Loss of vision	50 mm
	5	M/77 YO	Loss of vision	110 mm
	6	F/60 YO	Eye pain	150 mm
	7	F/55 YO	Loss of vision	110 mm
	8	F/50 YO	Loss of vision	100 mm
	9	M/50 YO	Loss of vision	125 mm
Angiostrongyliasis	1	M/23 YO	Dimness, bulge	22 mm
Thelaziasis	1	M/26 YO	Bulge-sticking pain	10-15 mm
	2	M/7 MO	Loss of vision	9-12 mm
	3	F/50 YO	Bulge-sticking pain	11-15 mm
Gnathostomiasis	1	M/39 YO	Loss of vision	6 mm

Table 1: The main information of ocular helminthiasis.

Note: Sex/age: *M = Male; F = Female; YO = Year old; MO = Month old.

Toxocariasis

Toxocariasis is widely distributed throughout the country [7]. This parasite can infect many places of the body, including the eyes. One case involved a 34-year-old man residing in the north of Vietnam who visited the National Eye Hospital (NEH) in August 2011. He felt pain from a bulge in his left eye and a loss of vision occurring over 3 months before visiting the hospital. The eye examination in the hospital showed damage of the left eye, red eye, retinal fibrosis, retinal detachment, inflammation of the eye tissues, retinal granulomas, and a parasitic cyst inside. Larva of *Toxocara* was collected by a medical doctor with surgery. Comparison of 264 nucleotides of internal transcribed spacer 2 (ITS2) of ribosomal DNA was performed between Vietnamese *Toxocara canis* and other *Toxocara* geographical isolates including Chinese *T. canis*, Japanese *T. canis*, Sri Lankan *T. canis*, and Iranian *T. canis*. The nucleotide homology was 97-99% when the Vietnamese *T. canis* was compared with other *T. canis* isolates worldwide. In the phylogenetic tree, Vietnamese *Toxocara canis* is in one group with strains of *Toxocara canis* in GenBank (Figure 1). Identification of *T. canis* infection in the eye by molecular methods was performed for the first time in Vietnam [8, 9].

Dirofilariasis

Dirofilariasis is uncommon in Vietnam [7], but from 2006 to 2010, hospitals in Hanoi detected 10 patients for dirofilariasis, including 9 ocular helminths. The worms were collected from the human eyes and identification of the species was completed. Nine parasites were recovered either from the conjunctiva of the eyes before species identification was conducted. Parasites were 4 - 12.5 cm in length and 0.5 - 0.6 mm in width. Morphological observation identified all parasites as *Dirofilaria repens* (Figure 2). Three of the 9 parasites were used for molecular-based confirmation of species identification. A portion of the mitochondrial *cox1* (461 bp) gene was amplified and sequenced for taxonomic analysis. Nucleotide and amino acid homology were 95% and 99-100%, respectively, when compared with *Dirofilaria repens* (of Italian origin, GenBank AJ271614; DQ358814). Vietnamese *Dirofilaria repens* is present in one group with strains of GenBank *Dirofilaria repens* in the phylogenetic tree (Figure 3).

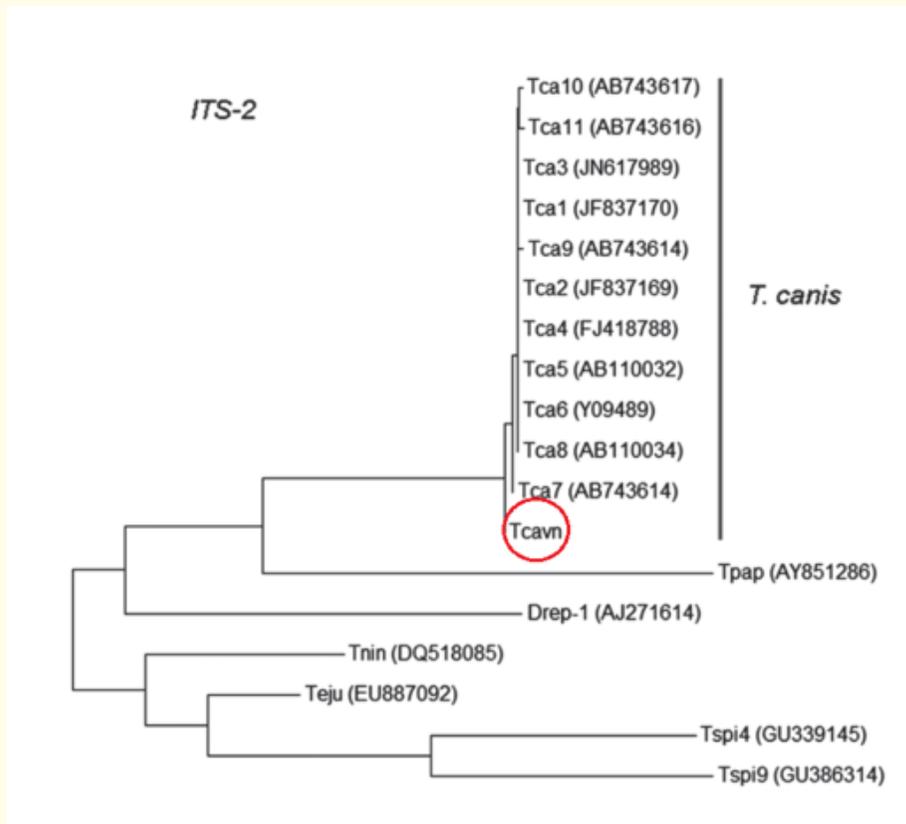


Figure 1: Phylogenetic tree of Vietnamese *Toxocara canis* and other isolates based on ITS-2 nucleotide sequence as estimated by neighbor-joining (NJ) using MEGA5 (Tamura et al., 2004). Tcavn = Vietnamese *T. canis*; Tca1, Tca2, and Tca3=Chinese *T. canis*; Tca4=Sri Lankan *T. canis*; Tca5 and Tca8=Japanese *T. canis*; Tca6=unknown *Toxocara*; Tca7, Tca9, Tca10, and Tca11= Iranian *T. canis*; Tpap=*Trichinella papuae* (GenBank no. AY851286); Teju=*Troglosiro cf. juberthiei* (GenBank no. EU887092); Tnin=*Troglosiro ninqua* (GenBank no. DQ518085); Tspi4=Chinese *Trichinella spiralis* (GenBank number GU339145); Tspi9=GenBank *Trichinella* (GenBank no. GU386314); Drep-1=Italian *Dirofilaria repens* (GenBank no. AJ271614) [12].

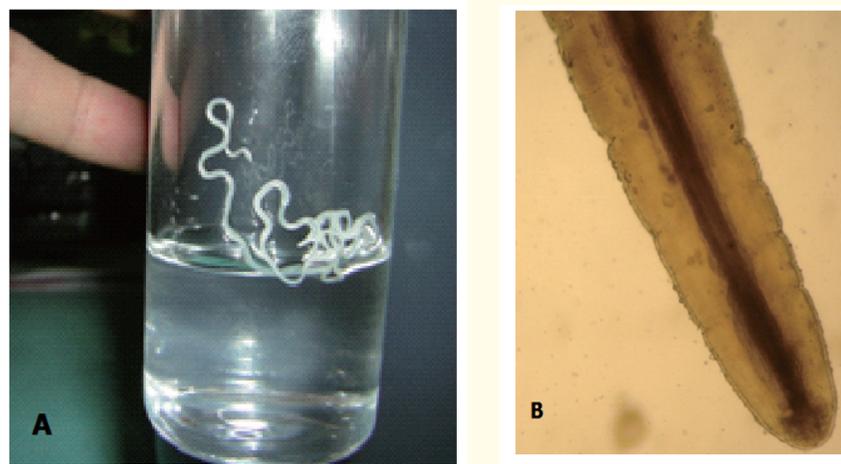


Figure 2: *Dirofilaria repens* collected from the conjunctiva (A) and head of the worm (B) of humans in Vietnam.

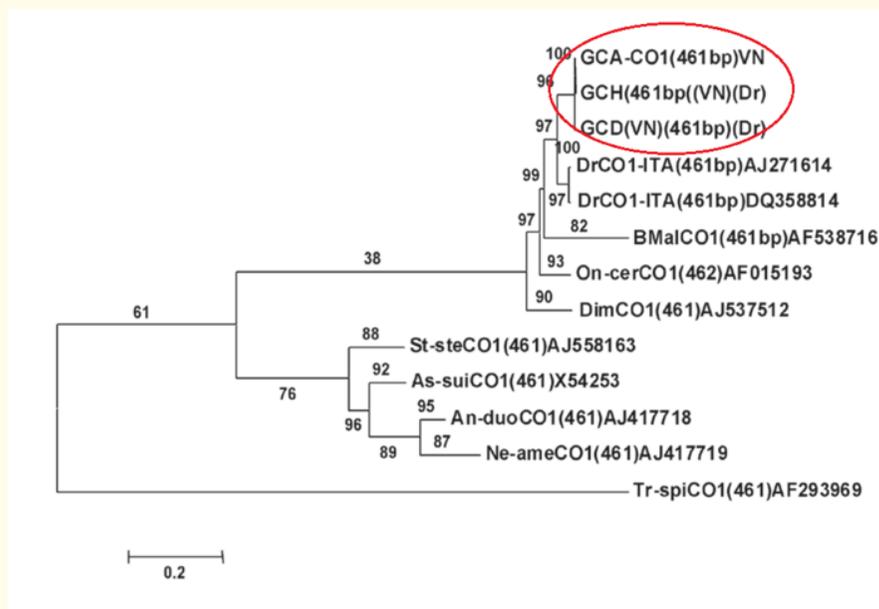


Figure 3: The phylogenetic tree based on a portion of the *cox1* sequence of *Dirofilaria* isolates and nematode strains, including 3 collected in Vietnam. Topology was constructed by MEGA 4.1 using the neighbor-joining method. *Dirofilaria repens* from this study and 2 from Italy are shown by the vertical bar. The length of the *cox1* sequence is indicated in brackets. Bootstrap values (%) are indicated in numerals from 1,000 replicates. GCA-CO1(461bp)VN, GCH(461bp)(VN)(Dr), and GCD(VN)(461bp)(Dr) = Vietnamese *Dirofilaria*; DrCO1-ITA(461bp)AJ271614 and DrCO1-ITA(461bp)DQ358814 = Italian *Dirofilaria repens* (GenBank no. AJ271614 and DQ358814); DimCO1(461) = *Dirofilaria immitis* (GenBank no. AJ537512); BmalCO1(461bp) = *Brugia malayi* (GenBank no. AF538716); On-cerCO1(462) = *Onchocerca volvulus* (GenBank no. AF015193); St-steCO1(461) = *Strongyloides stercoralis* (GenBank no. AJ558163); As-suiCO1(461) = *Ascaris sum* (GenBank no. X54253); An-duoCO1 = *Ancylostoma duo-denale* (GenBank no. AJ417718); Ne-ameCO1(461) = *Necator america-nus* (GenBank no. AJ417719); Tr-spiCO1(461) = *Trichinella spiralis* (GenBank no. AF293969) [13].

Angiostrongyliasis

Angiostrongyliasis is distributed in many Vietnamese provinces, especially in children [7]. Most of these nematodes parasitize the brain, but they may be moving in the eye [7,11]. One case involved a 23-year-old female residing in the north of Vietnam, who visited the Hanoi Medical University Hospital in July 2013. She felt a dimness in the eyes and pain from a bulge in her left eye for some days before visiting the hospital. While at the hospital, a clinical examination, an eye endoscopy and an operation were performed. A nematode worm was collected from the eye of this patient. The body of this worm was thin and long and measured 22 x 0.3 mm (Figure 4). By a molecular method using the 18S rRNA gene, this worm was identified as *Angiostrongylus cantonensis*. The phylogenetic tree showed that Vietnamese *Angiostrongylus cantonensis* is present in one group with strains of GenBank *Angiostrongylus cantonensis* (Figure 5). This is the first molecular study involving identification of *Angiostrongylus cantonensis* in Vietnam [12]. In 2002 the first case of *Ocular Angiostrongylus cantonensis* was identified in a female Vietnamese patient but was only described by morphological characterization [13].

Thelaziasis

Thelaziasis is uncommon in Vietnam, and at present three cases have been identified, including a 26-year-old man, a 7-month-old male child, and a 50-year-old woman in the north mountainous area of Vietnam. These nematodes were identified based on morphological characterization (Figure 6) and identified as *Thelazia callipaeda* by a molecular method using Cox1 with 99% homology compared with *Thelazia callipaeda* in GenBank. The phylogenetic tree of *Thelazia callipaeda* Vietnam and other strains determined from part of the Cox1 nucleotide sequence estimated by Neighbor-Joining (NJ) using MEGA5.1 (Tamura, et al. 2004) showed that the strain of Vietnamese *Thelazia callipaeda* is present in one group with strains of *Thelazia callipaeda* in GenBank (Figure 7). The present study is the first to report the presence of human *T. callipaeda* infection in Vietnam [14, 15].



Figure 4: An immature female worm of *Angiostrongylus cantonensis* collected from the human eye in Vietnam.

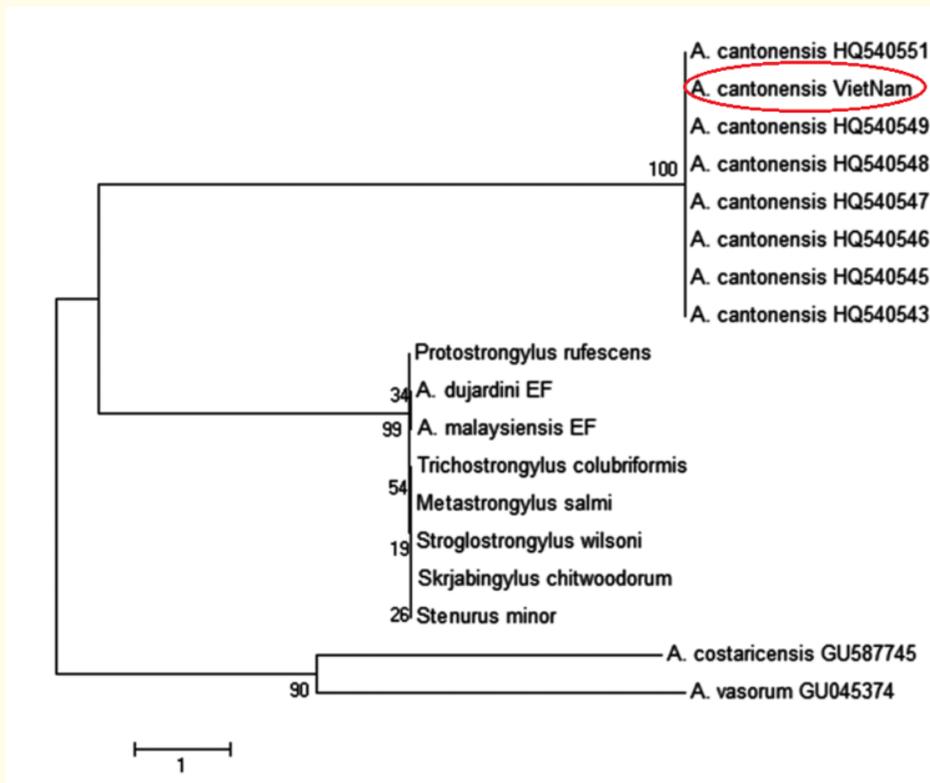


Figure 5: Phylogenetic tree of *Angiostrongylus cantonensis* Vietnam and other isolates targeting a part of 18S rRNA nucleotide sequences estimated by the neighbor-joining (NJ) using MEGA4.0 [9]. Note: *A. cantonensis* Vietnam = Vietnamese *Angiostrongylus*; *A. cantonensis* – GenBank no. HQ540548; *A. cantonensis* – GenBank no. HQ540547; *A. cantonensis* – GenBank no. HQ540546; *A. cantonensis* – GenBank no. HQ540545; *A. cantonensis* – GenBank no. HQ540543; *A. cantonensis* – GenBank no. HQ540551; *A. cantonensis* – GenBank no. HQ540549; *A. dujardini* EF – GenBank no. EF514915; *A. malaysiensis* EF – GenBank no. EF514914; *Metastrongylus salmi* – GenBank no. AY295809; *Protostrongylus rufescens* – GenBank no. AJ920364; *Skrjabinigylus chitwoodorum* – GenBank no. AY295819; *Stenurus minor* – GenBank no. AY295817; *Stroglostrogylus wilsoni* – GenBank no. AY295820; *Trichostrongylus colubriformis* – GenBank no. AJ920350; *A. costaricensis* – GenBank no. GU587745; *A. vasorum* – GenBank no. GU045374 [15].

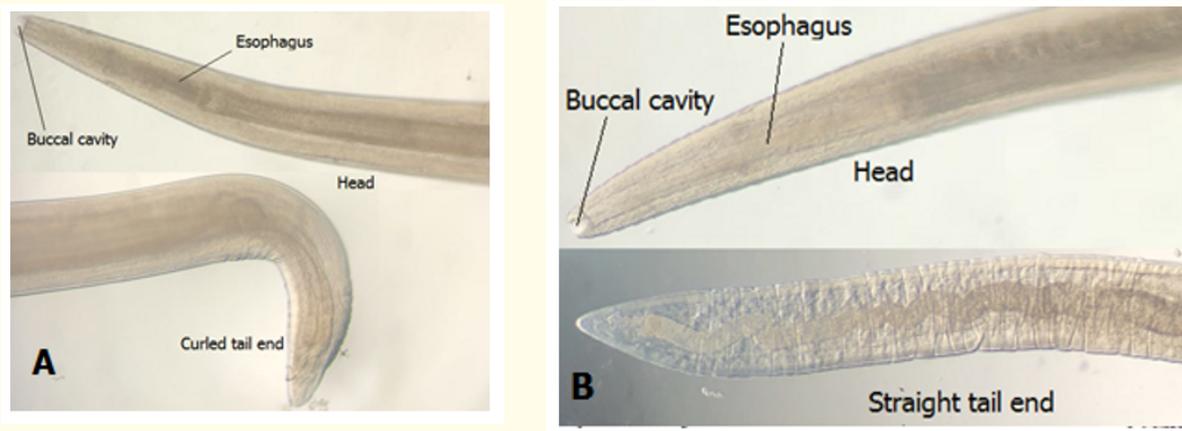


Figure 6: Morphology of the male *Thelazia callipaeda* (A) and female (B) from Vietnamese patients [18].

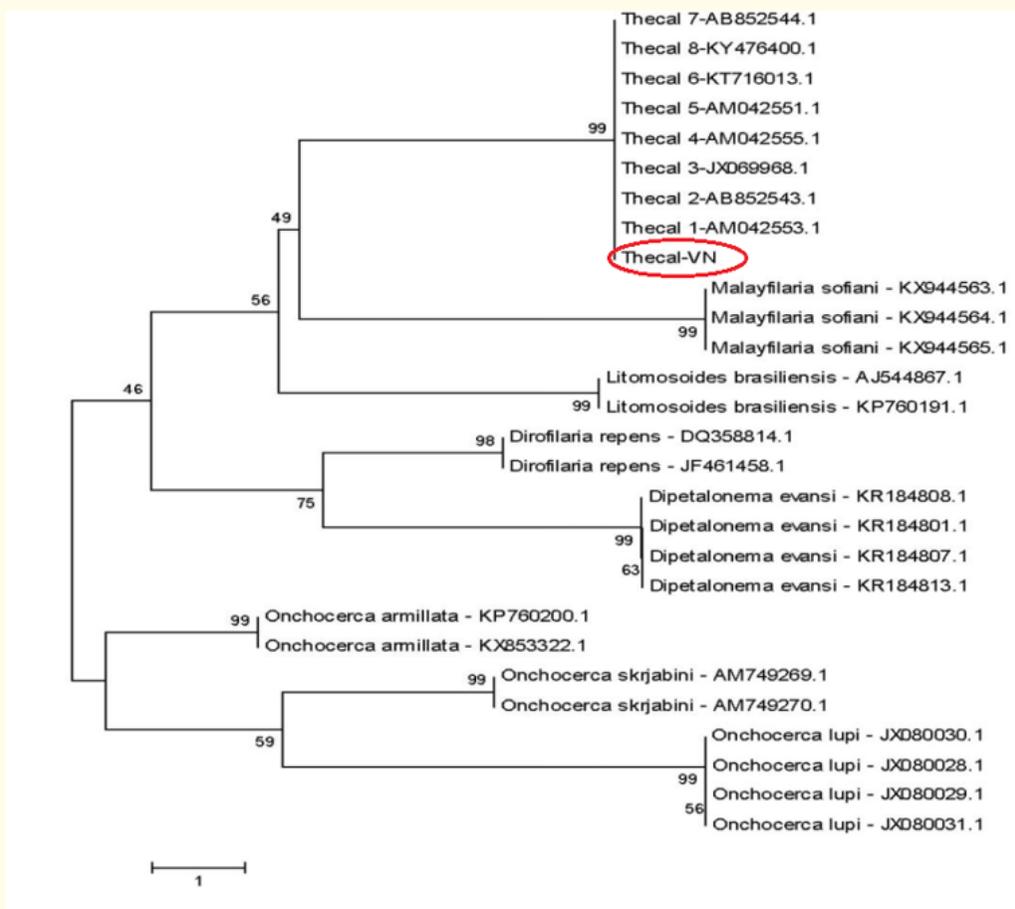


Figure 7: Phylogenetic tree of *Thelazia callipaeda* Vietnam and other strains from *cox1* gene nucleotide sequence estimated by Neighbor-Joining (NJ) using MEGA5.4 (Tamura et al., 2004). Note: Thecal-VN = Vietnamese *Thelazia*; other *T. callipaeda* (GenBank number: AM042553.1, AB852543.1, JX069968.1, AM042555.1, AM042551.1, KT716013.1, AB852544.1, and KY476400.1 respectively). *Malayfilaria sofiani* are from Malaysia (GenBank number: KX944563.1, KX944564.1, and KX944565.1), *Litomosoides brasiliensis* are from Brazil (GenBank number: AJ544867.1 and KP760191.1). *Dirofilaria repens* (GenBank number: DQ358814.1 and JF461458.1). *Dipetalonema evansi* are from Iran (GenBank number: KR184801.1, KR184807.1, KR184808.1, and KR184813.1). *Onchocerca armillata* (GenBank number: KP760200.1 and KX853322.1). *Onchocerca skrjabini* are from Japan (GenBank number: AM749269.1 and AM749270.1). *Onchocerca lupi* are from USA (GenBank number: JX080028.1, JX080029.1, JX080030.1, and JX080031.1) [18].

Gnathostomiasis

Gnathostomiasis is distributed in every province in Vietnam. A 39-year-old male Vietnamese patient was admitted to Cho Ray Hospital, Ho Chi Minh City. The patient felt pain from a bulge in his right eye, irritation and blurred vision for one month. About two months previously, he had suffered from migratory swellings on the right side of his face, then to the right cheek and then on the chin and upper lip. Later, an itch developed in his right eye and the area around the eye became swollen. One month later, he developed redness of and photophobia in the affected eye and a gradual decrease in visual acuity. A worm, red-orange in colour, was collected from the eye and it measured approximately 6 mm in length. The morphology of the hooklets and the presence of body spines of the larva were examined under a light microscope. The head bulb had four rows of hooklets and the number of hooklets were 43, 47, 49 and 51 (Figure 8). It was determined to be a third-stage *Gnathostoma spinigerum*. This worm was identified as *Gnathostoma spinigerum* by molecular method using the ITS-2 gene and compared with *Gnathostoma spinigerum* from Thailand (KP784342) and Laos (KP784336). The results showed that the homology of nucleotides between Vietnamese *Gnathostoma* and *Gnathostoma spinigerum* from Thailand and Laos was 99.4%. However, Vietnamese *Gnathostoma* was identified as *Gnathostoma spinigerum* [16]. In the phylogenetic tree, Vietnamese *Gnathostoma spinigerum* is present in one group with *Gnathostoma spinigerum* from Laos and Thailand (Figure 9).

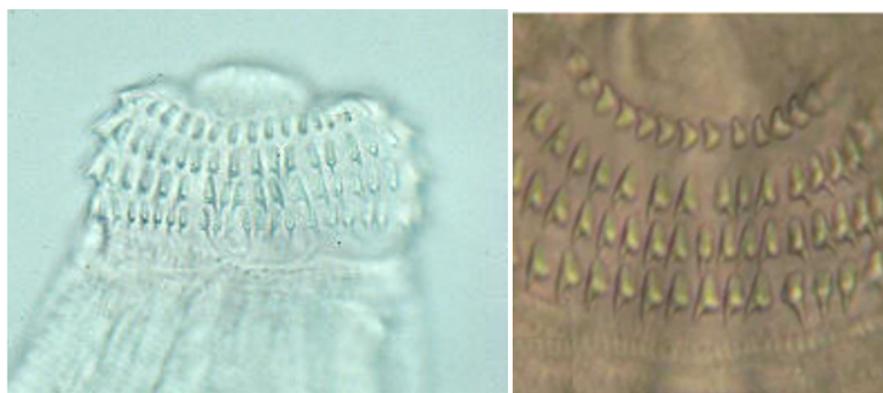


Figure 8: *Gnathostoma spinigerum* larvae in the study.

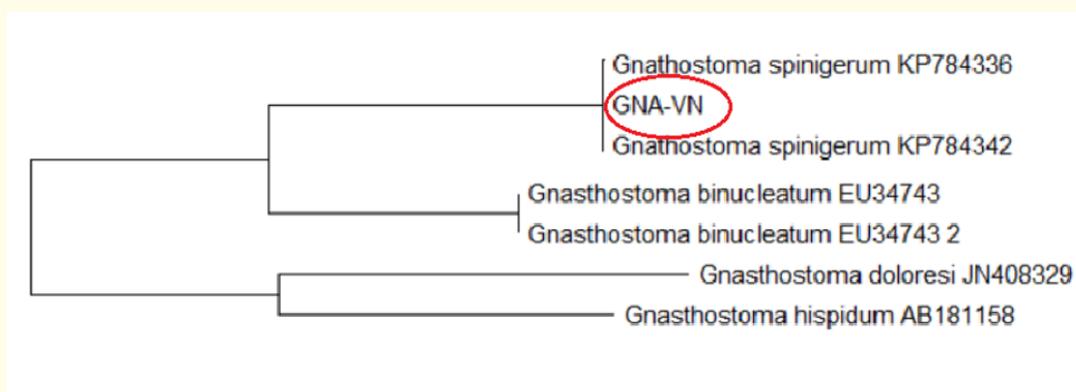


Figure 9: Phylogenetic relationships of the Vietnamese *Gnathostoma* and other representative species in the world. Note: GNA-VN = Vietnamese *Gnathostoma*; KP784336 = *Gnathostoma spinigerum* from Laos; KP784342 = *Gnathostoma spinigerum* from Thailand and others from GenBank.

Discussion

The ocular toxocariasis was reported in Vietnam and this case was similar to the report of John and Petri in their Review of Optometry Online, Handbook of Ocular Disease Management [1].

In this study, 9 *Dirofilaria* worm specimens from human eyes, from the conjunctiva were identified by morphological and molecular methods as with *D. repens*. In Vietnam also detected two cases of *Dirofilaria repens* in subcutaneous tissue [10]. There have been over 1,000 cases of dirofilariasis reported worldwide, including 300 cases involving the lungs or viscera, and over 800 cases involving subcutaneous tissues or the eyes [3].

Three human cases of thelaziasis were reported in Vietnam including a 26-year-old man, a 7-month-old child and a 50-year-old woman, and 5, 7 and 8 small worms were collected, respectively, from the eyes. Molecular methods involving sequencing of a portion of the cytochrome C oxidase subunit 1 (cox1) gene of different *Thelazia callipaeda* species from GenBank and compared with *Thelazia callipaeda* in Vietnam were performed. Results showed that the nucleotide homology between the Vietnamese isolate and 8 other isolates worldwide was 98% ~ 99% [1, 14, 15].

The parasitic site of *Angiostrongylus* has usually been the brain, but can be in other places, including the eyes. In this report, a worm was collected from the eye. In 2001, Xuan., *et al.* [17] also reported a case involving infection of the eye with *A. cantonensis*. Feng., *et al.* [18] reviewed a total of 42 ocular cases reported worldwide.

A case of ocular *Gnathostoma spinigerum* was reported in Vietnam using the morphology-based and molecular method [16]. To date, 74 cases of intraocular gnathostomiasis have been reported from 12 countries worldwide [6].

Conclusion

Five species of ocular helminths were reported in Vietnam including *Toxocara canis*, *Dirofilaria repens*, *Angiostrongylus cantonensis*, *Thelazia callipaeda*, and *Gnathostoma spinigerum*. These worms were collected from the eyes and identification was based on morphological and molecular methods. Most of these were reported for the first time in Vietnam.

Conflicts of Interest

The authors report no conflicts of interest.

Acknowledgments

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Bibliography

1. John DT and Petri WA. "Markell and Voge's Medical Parasitology". 9th ed. St. Louis, Missouri, USA. Saunders Elsevier (2006).
2. Malla N and Goyal K. "Ocular Parasitic Infections – An Overview, Advances in Common Eye Infections". *Shimon Rumelt - IntechOpen Open Access* (2016).
3. Pampiglione S and Rivasi F. "Human dirofilariasis due to *Dirofilaria* (Nochtiella) *repens*: An update of world literature from 1995 to 2000". *Parasitol* 42 (2000): 231-254.
4. Otranto D and Eberhard ML. "Zoonotic helminths affecting the human eye". *Parasit Vectors* 4 (2011): 41.
5. Nimir AR., *et al.* "Ophthalmic parasitosis: a review article". *Interdisciplinary Perspectives on Infectious Diseases* 2012 (2012): 587402.
6. Nawa Y., *et al.* "Ocular Gnathostomiasis: A Comprehensive Review". *The Journal of Tropical Medicine and Parasitology* 33 (2010): 77-86.
7. De NV., *et al.* "Medical Parasites. Education Book for Medical Doctors". Hanoi: Academic Press. (2016): 322-335.
8. De NV., *et al.* "Clinical symptoms of human toxocariasis and identification species of *Toxocara* sp". *Journal of Malaria and Parasitic Diseases Control* 4 (2014): 25-30.

9. De NV., et al. "Molecular Diagnosis of an Ocular Toxocariasis Patient in Vietnam". *The Korean Journal of Parasitology* 51.55 (2013): 1-5.
10. De NV., et al. "Dirofilaria repens in Vietnam: detection of 10 cases of the eyes and subcutaneous tissue dirofilariasis, identification of species by morphological and molecular method". *The Korean Journal of Parasitology* 50,2 (2012): 137-141.
11. De NV. "Report of eosinophilia encephalic caused by Angiostrongylus". *Journal of Medical pharmaceutical information* 5 (2004): 12-15.
12. De NV., et al. "A Case of Ocular Angiostrongyliasis with Molecular Identification of the Species in Vietnam". *The Korean Journal of Parasitology* 53.6 (2015): 713-717.
13. Thu TP., et al. "Ocular Angiostrongylus cantonensis in a female Vietnamese patient: case report". *Klin Monbl Augenheilkd* 219.12 (2002): 892-895.
14. De NV., et al. "The First Case of Thelazia callipaeda Infection in Vietnam". *The Korean Journal of Parasitology* 50.3 (2012): 221-223.
15. De NV., et al. "Detection of Two Patients, who were Infected by Eye Thelazia callipaeda in Vietnam 2017". *EC Ophthalmology* 9.11 (2018): 733-739.
16. Xuan LT., et al. "Case report: intraocular gnathostomiasis in Vietnam". *The southeast asian journal of tropical medicine and public health* 33.3 (2002): 485-489.
17. Xuan LT., et al. "A case infected by Angiostrongylus cantonensis in the eye in Ho Chi Minh City". *J Medicine Ho Chi Minh City* 1 (2001): 97-100.
18. Feng Y., et al. "Comprehensive review of ocular angiostrongyliasis with special reference to op-tic neuritis". *The Korean Journal of Parasitology* 51 (2013): 613-619.

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