Tropical Biomedicine 36(2): 475–481 (2019)

Dirofilariasis in Vietnam: A case report and brief review

Que, A.T.[1], Nguyen, D.N.[2], Do, N.A.[3] and Le, T.A.[3*]
[1]Department of Tropical disease, Vinh Hospital of Frienship General, Lenin boulevard, Vinh, Nghe An, Vietnam
[2]Department of Ophthalmology, 103 hospital, Vietnam Military Medical University, Phung Hung, Ha Dong, Ha Noi, Vietnam
[3]Department of Parasitology, Vietnam Military Medical University, Phung Hung, Ha Dong, Ha Noi, Vietnam
[*]Corresponding author e-mail: anh_lt@vmmu.edu.vn
Received 18 December 2018; received in revised form 26 February 2019; accepted 27 February 2019

Abstract. This report describes a rare case of ophthalmic dirofilariasis in a 68-year-old woman with red and foreign body sensation in the pterygium on her right eye. Slit lamp examination demonstrated a long-slender worm moving in her pterygium. The worm was removed surgically and then identified as *Diroflaria repens* by sequence analysis of the small subunit ribosomal RNA (SSU) gene. The situation of dirofilariasis in Vietnam has been reviewed. Since the first described case in 2010 there have been thirteen cases reported that suggested the emerging trend of the disease. Most of the documented cases of human dirofilariasis recorded in Vietnam presented with ocular infections and the responsible agent was *D. repens*. With the increase of reported cases of human, much more attention should be paid on control as well as diagnosis and treatment of dirofilariasis in Vietnam.

INTRODUCTION

Dirofilariasis is a zoonotic disease caused by *Dirofilaria* worm infections. At least six species of *Dirofilaria* have been reported to cause human infections including *Dirofilaria immitis*, *Dirofilaria repens*, *Dirofilaria striata*, *Dirofilaria tenuis*, *Dirofilaria ursi* and *Dirofilaria spectans* (Reddy, 2013). The main natural host for those species is domestic dogs and some other less suitable hosts such as cats, foxes, wolves (Joseph *et al.*, 2011). The worms are transmitted to humans via mosquito bites. Females of various mosquito species of the *Culicidae* family can transmit *Dirofilaria* infections to humans (Cancrini & Kramer, 2001). Human dirofilariasis is thought to be rare, but more and more cases have been reported and is considered an emerging disease (Pampiglione & Rivasi, 2000), (Simón *et al.*, 2012). The distribution of species of *Dirofilaria* may vary in different regions, and *D. repens* along with *D. immitis* are considered the most important agents regarding the number of cases reported and the wide geographical distribution (Simón *et al.*, 2012). The most common disease caused by *D. immitis* are pulmonary dirofilariasis while those by *D. repens* are subcutaneous or ophthalmic dirofilariasis (Simón *et al.*, 2012).

In Vietnam, since the first reported infection in 2010 (Dang *et al.*, 2010), cases of dirofilariasis are more and more reported (De *et al.*, 2012), (Le *et al.*, 2015) suggesting the emerging trend of the disease. However, little is known about epidemiological characteristics of the infection such as species distribution or who are the most affected group(s). Here we report a case of *D. repens* infection and review the situation of dirofilariasis in Vietnam.

CASE REPORT

On 31 May 2018, a 68-year-old woman presented to an ophthalmologist because of redness and a foreign body sensation in the pterygium on her right eye. During the two days before the admission, she had felt the swelling of nasal palpebral fissure and something slowly moving under the nasal conjunctiva which caused much annoyance. The slit-lamp examination showed a long-slender worm moving in her pterygium (Fig. 1). The patient underwent surgical removal of the worm (Fig. 2) and recovered completely after treatment by antibiotic and anti-inflammatory solutions.

To identify the worm total genomic DNA was extracted from an ethanol-fixed fragment of the excised worm by QIAamp DNA Mini kit (QIA-GEN, Hilden, Germany) according to manufacturer's instructions. PCR of a small subunit ribosomal RNA

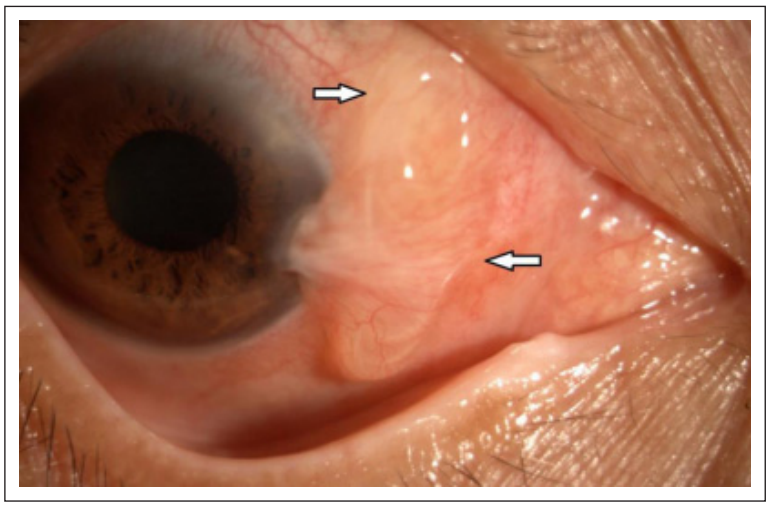

Figure 1. The worm (arrow) in pterygium of patients' right eye.

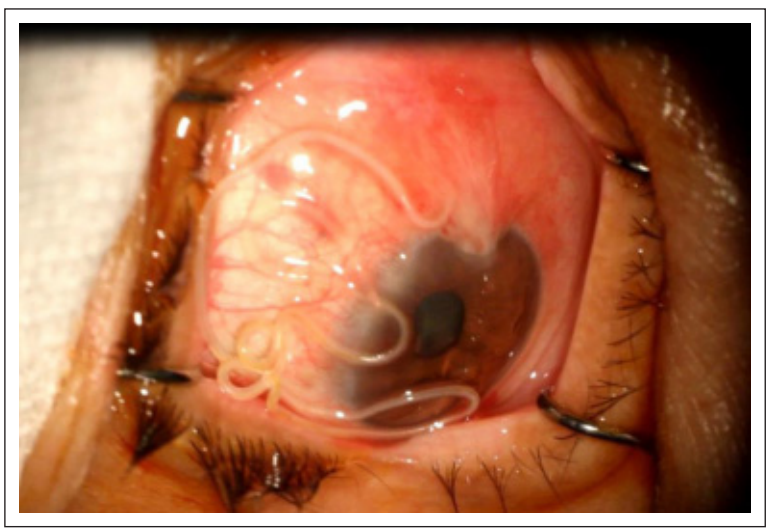

Figure 2. The operation to collect the worm.

Table 1. Thirteen cases of human dirofilariasis in Vietnam

N	Gender	Age	Location	Agent	Residence	Year	Reference
1	Female	30	Eyelid	*D. repens*	Ha Noi	2008	Dang *et al.*, 2010
2	Female	50	Sub-conjunctiva	*D. repens*	Ha Noi	2006–2010	De *et al.*, 2012
3	Male	47	Sub-conjunctiva	*D. repens*	Ha Noi	2006–2010	De *et al.*, 2012
4	Female	27	Sub-conjunctiva	*D. repens*	Ha Noi	2006–2010	De *et al.*, 2012
5	Male	49	Sub-conjunctiva	*D. repens*	Ha Noi	2006–2010	De *et al.*, 2012
6	Male	77	Sub-conjunctiva	*D. repens*	Ninh Binh	2006–2010	De *et al.*, 2012
7	Female	60	Sub-conjunctiva	*D. repens*	Ninh Binh	2006–2010	De *et al.*, 2012
8	Female	55	Sub-conjunctiva	*D. repens*	Hung Yen	2006–2010	De *et al.*, 2012
9	Female	50	Sub-conjunctiva	*D. repens*	Hung Yen	2006–2010	De *et al.*, 2012
10	Male	50	Sub-conjunctiva	*D. repens*	Ha Nam	2006–2010	De *et al.*, 2012
11	Male	36	Subcutaneous	*D. repens*	Ha Nam	2006–2010	De *et al.*, 2012
12	Female	34	Subcutaneous	*D. repens*	Ha Noi	2011	Le *et al.*, 2015
13	Female	64	Sub-conjunctiva	*D. repens*	Ha Noi	2018	This case

(SSU) gene was performed using the primer pairs (forward SSU18A, 5´(5'-AAA GAT TAA GCC ATG CAT G-3') and reverse SSU26R, (5'-CAT TCT TGG CAA ATG CTT TCG-3') (Floyd *et al.*, 2002). The components of the PCR reaction were as follows: 5 µl of 10X PCR buffer, 2 mM MgCl2, 5 µl of 2 mM dNTPs (0.2 mM of each), 0.3 µM each primer, 1.25 units of Taq polymerase (Thermo Scientific, USA), 5 µl of template DNA and molecular grade dH_2O up to 50 µl. Reaction mixtures were subjected to initial denaturation at 94°C for 4 min, then 35 cycles including denaturation at 94°C for 45 sec, annealing at 58°C for 45 sec, extension at 72°C for 1 min; and a final cycle of 10 min at 72°C to obtain a PCR product. The product of PCR reaction was sequenced and analyzed by BLAST tool showing 99% identity with the SSU gene of *D. repens*. The sequence was deposited in the GenBank under accession number MH981971.

Analysis of the reported cases of human dirofilariasis in Vietnam.

Up to now there have been thirteen cases (including the case in the current study) in Vietnam. Females composed 8 cases and male cases numbered 5. Eleven cases had lesions in the eye region and two cases had subcutaneous lesions. Only *D. repens* was identified in all 13 patients.

DISCUSSION

Demographic characteristics of the patients: among reported cases in Vietnam more females were affected than males (female/male = 1.6/1). There was a report of 72.5% of cases in women (L. Ermakova *et al.*, 2017). The patients were aged between 27 and 77, and this was in line with that in other reports showing that dirofilariasis mostly affected persons of adult age (L. Ermakova *et al.*, 2017).

Geographic distribution of patients: all reported patients lived in northern Vietnam and 7 out of 13 patients lived in Ha Noi, the capital city of Vietnam. In facts, all samples were collected from hospitals in Ha Noi and easily sent to institutes with available equipment for molecular analysis such as National Institute of Malariology, Parasitology and Entomology (NIMPE); Vietnam Military Medical University, Institute of Bio-Technology which are all located in Ha Noi. Ten worms in the report of De *et al.*, (2012) were only identified and reported with funds supported from the National Foundation for Science and Technology Development (NAFOSTED) in Vietnam. In our opinion this may represent the limited resources to perform laboratory diagnostic approaches including molecular identifi-

cation/discrimination of the agent but not the limited distribution of the infection in Vietnam.

Localization of human dirofilariasis: the localization of lesions varies among different species. *D. repens* localize mainly in the external parts of the body, subcutaneous (normally nodular) and the submucosal (nodular or not) (Pampiglione and Rivasi 2000), whilst human infections with *D. immitis* are mainly found in the lungs and present as pulmonary forms (Simón *et al.*, 2012). Among dirofilariasis cases in Vietnam, lesions are mostly localized in the eye region (11/13=84.62%) which is consistent with a previous report on the most common localization of human *D. repens* infections (L.A. Ermakova *et al.*, 2014). Ocular dirofilariasis can be presented as scleritis (Sangit and Haldipurkar 2012), periorbital and subconjunctival cysts, eyelid swelling (Nath *et al.*, 2010) and motile swelling on the conjunctiva (Patel *et al.*, 2014). In Vietnam, the most common involvement in ocular dirofilariasis was subconjunctival lesions (with 10/11 cases sub-conjunctiva and 1/11 was on the eyelid). Although *D. immitis* rarely affect eye regions, ocular dirofilariasis caused by *D. immitis* have been reported in Iran (Mirahmadi *et al.*, 2017), Italy (Avellis *et al.*, 2011). However, all cases in Vietnam were due to *D. repens*. The case reported in this study was also a woman with a lesion in her pterygium.

Diagnosis and treatment: most cases with lesions on sub-conjunctiva were suspected under clinical examination because the transparence of the conjunctiva, but all cases were definitively diagnosed only following surgery. There were no support tests for diagnosis of dirofilariasis in Vietnam. The definite diagnosis of dirofilariasis can be made by examination of histological sections of worms and/or of the infected tissue collected during surgery (ESDA, 2017). ELISA may be a complementary and reasonable diagnostic procedure with a diagnostic accuracy as high as 83% (L.A. Ermakova *et al.*, 2014). Nevertheless, reagents for the serological tests are not available in Vietnam due to the rarity of the disease. Imaging methods are only valuable in dirofilariasis with the involvement of internal organs (ESDA, 2017), but all reviewed cases were localized in sub-cutaneous and sub-mucosa regions.

The identification of species of *Dirofilaria* was based on the microscopy of removed parasites and molecular tools. In all cases, a morphological study of adult worms was first done to give an initial adjustment. Although morphological characteristics can be used to differentiate *D. repens* from *D. immitis* where external longitudinal cuticular ridges present in *D. repens* but not in *D. immitis* (Sathyan *et al.*, 2006). However, some other zoonotic *Dirofilaria* species share morphologic features with *D. repens* (Wong & Brummer, 1978) making identification of *Dirofilaria* spp. based only on morphology difficult. In all previous and the current study, molecular methods were used to confirm the identification of the species. Different targets have been used to identify the species of *Dirofilaria* in Vietnam including mitochondrial cytochrome c oxidase I (cox1) gene (De *et al.*, 2012), (Le *et al.*, 2015), 12S ribosomal RNA (12S rRNA) genes (Dang *et al.*, 2010), 18S ribosomal RNA (18S rRNA) genes (this report). The 16SRNA region of the endosymbiont *Wolbachia pipientis*, a Gram-negative bacteria commonly co-existing with *Dirofilaria* spp. (Casiraghi *et al.*, 2004) was sequenced to support the identification in one study (Le *et al.*, 2015). Those approaches have been applied in some reports (Kelvin K.W. To *et al.*, 2012), (Simsek & Ciftci, 2016), (Nazar *et al.*, 2017). Analysis of mitochondrial genome fragment showing that the genetic differences between the sample from Vietnam and Europe samples was larger than between Vietnam and some Asian samples (Indian and Thai samples) (Yilmaz *et al.*, 2016).

Species: all 13 reported cases of dirofilariasis in Vietnam were caused by *D. repens* and no case of *D. immitis* infection is known. This result was consistent with a review by Simón F. *et al.* (2012) finding cases of human infection with *D. immitis* to be dominant in the Americas

and Australia, while in Asia most cases reported were caused by *D. repens*, except for Japan where *D. immitis* was more prevalent (Simón *et al.*, 2012). Because of the lack of data of *Dirofilaria* infection in animals, the risk of human infection with *D. immitis* in Vietnam has been an unanswered question. With the sporadic pulmonary dirofilariasis cases attributed to *D. immitis* in some neighboring countries such as Thailand (Sukpanichnant *et al.*, 1998) and Taiwan (Tsung & Liu, 2003), and the difficulty in diagnosis of pulmonary dirofilariasis due to the small size of the lesions in the lung (Sukpanichnant *et al.*, 1998), there may be any number of undiagnosed dirofilariasis caused by *D. immitis* in Vietnam.

Potential vectors: some other studies have proved the role of *Anopheles maculipennis*, *Aedes aegypti*, *Aedes albopictus*, *Mansonia uniformis*, *Mansonia annulifera* and *Armigeres obturbans* as vectors transmitting *D. repens* (Simón *et al.*, 2012). Although there have been no studies of vectors for dirofilariasis in Vietnam many species of above mosquitoes were distributed in many parts of Vietnam and responsible for some other vector-borne diseases such as Dengue virus infection, encephalitis, filariasis (Higa *et al.*, 2010), (Ohba *et al.*, 2015).

CONCLUSION

With the increased report of cases of human dirofilariasis in Vietnam, information involving epidemiological characteristics namely etiological species, animal/host reservoirs, vectors/mosquitoes and spatial distribution is needed. Clinicians must be aware of the existence of dirofilariasis and include this disease in different diagnosis for routine practice. The improvement of laboratory approaches including molecular identification of *Dirofilaria* is necessary for early diagnosis of emerging dirofilarial infections. Vector management should be enhanced in integration with control of some viral diseases which are prevalent in Vietnam such as Dengue virus infection, encephalitis and transmitted to human through mosquitoes of *Culicidae* family.

Declarations

Ethics Approval and Consent to Participate

Written informed consent was obtained from the patient for the publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Consent for publication

The authors agreed to publish this article.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Funding

Not applicable.

Authors' contributions

TAL and ATQ designed the study; NDN collected the clinical data and did the surgery, NAD and TAL identified species of the responsible agent. ATQ and TAL drafted the manuscript. All authors read and approved the final manuscript.

REFERENCES

Avellis, F.O., Kramer, L.H., Mora, P., Bartolino, A., Benedetti, P. & Rivasi, F. (2011). A Case of Human Conjunctival Dirofilariosis by Dirofilaria Immitis in Italy. *Vector Borne Zoonotic Disease* **11**: 451-52.

Cancrini, G. & Kramer, L. (2001). Insect Vectors of Dirofilaria spp. In: *Heartworm Infection in Humans and Animals*, eds. F Simón and C Genchi. Salamanca, Spain: Ediciones Universidad de Salamanca, pp. 63-82.

Casiraghi, M., Bain, O., Guerrero, R., Martin, C., Pocacqua, V., Gardner, S.L., Franceschi, A. & Bandi, C. (2004). Mapping the Presence of Wolbachia Pipientis on the Phylogeny of Filarial Nematodes: Evidence for Symbiont Loss during Evolution. *International Journal for Parasitology* **34**: 191-203.

Dang, T.C., Nguyen, T.H., Do, T.D., Uga, S., Morishima, Y., Sugiyama, H., Yamasaki, H. (2010). A Human Case of Subcutaneous Dirofilariasis Caused by Dirofilaria Repens in Vietnam/: Histologic and Molecular Confirmation. *Parasitology Research* **107**: 1003-7.

De, Nguyen Van, Thanh Hoa Le & Jong-yil Chai. (2012). Dirofilaria Repens in Vietnam/: Detection of 10 Eye and Subcutaneous Tissue Infection Cases Identified by Morphology and Molecular Methods. *The Korean Journal of Parasitology* **50**: 137-41.

Ermakova, L., Nagorny, S., Pshenichnaya, N., Ambalov, Y. & Boltachiev, K. (2017). Clinical and Laboratory Features of Human Dirofilariasis in Russia. *ID Cases* **9**: 112-15.

Ermakova, L.A., Nagorny, S.A., Krivorotova, E.Y., Pshenichnaya, N.Y. & Matina, O.N. (2014). Dirofilaria Repens in the Russian Federation: Current Epidemiology, Diagnosis, and Treatment from a Federal Reference Center Perspective. *International Journal of Infectious Diseases* **23**: 47-52.

European Society of Dirofilariosis and Angiostrongylosis. (2017). *Guidelines for Clinical Management of Human Dirofilaria Infections Prepared for and Approved by the Executive Board of ESDA*.

Floyd, Robin, Eyualem Abebe, Artemis Papert & Mark Blaxter. (2002). Molecular Barcodes for Soil Nematode Identification. *Molecular Ecology* **11**: 839-50.

Joseph, Elizabeth, Anna Matthai, Latha K. Abraham & Sunitha Thomas. (2011). Subcutaneous Human Dirofilariasis. *Journal of Parasitic Diseases* **35**: 140-43.

Higa, Y., Yen, N.T., Kawada, H., Son, T.H., Hoa, N.T. & Takagi, M. (2010). Geographic Distribution of Aedes Aegypti and Aedes Albopictus Collected from Used Tires in Vietnam. *Journal of the American Mosquito Control Association* **26**: 1-9.

Kelvin, K.W. To, Samson S.Y. Wong, Rosana W.S. Poon, Nigel J. Trendell-Smith, Antonio H.Y. Ngan, Jacky W.K. Lam, Tommy H.C. Tang, Ah-Kian Ah Chong, Joshua Chi-Hang Kan, Kwok-Hung Chan & Kwok-Yung Yuen. (2012). A Novel Dirofilaria Species Causing Human and Canine Infections in Hong Kong. *Journal of Clinical Microbiology* **50**: 3534-3541.

Le, Tran Anh, Thuat Thang Vi, Khac Luc Nguyen & Thanh Hoa Le. (2015). A Rare Human Case of Dirofilaria Repens Infection in the Subcutaneous Posterior Thorax with Molecular Identification. *The Korean Journal of Parasitology* **53**: 329-33.

Mirahmadi, Hadi, Alireza Maleki, Raheleh Hasanzadeh & Mohammad Bagher. (2017). Ocular Dirofilariasis by Dirofilaria Immitis in a Child in Iran: A Case Report and Review of the Literature. *Parasitology International* **66**: 978-81.

Nath, R., Gogoi, R., Bordoloi, N. & Gogoi, T. 2010). Ocular Dirofilariasis. *Indian Journal of Pathology and Microbiology* **53**: 157-59.

Nazar, Najuma, Bindu Lakshmanan, Jayavardhanan, K.K. (2017). Molecular Characterization of Human Dirofilaria Isolates from Kerala. *The Indian Journal of Medical Research* **146**: 528-33.

Ohba, S.Y., Van Soai, N., Van Anh, D.T., Nguyen, Y.T. & Takagi, M. (2015). Study of Mosquito Fauna in Rice Ecosystems around Hanoi, Northern Vietnam. *Acta Tropica* **142**: 89-95.

Pampiglione, S. & Rivasi, F. (2000). Human Dirofilariasis due to Dirofilaria (Nochtiella) Repens: An Update of World Literature from 1995 to 2000. *Parassitologia* **42**: 231-54.

Patel, R., Singh, S. & Bhavsar, S. (2014). A Rare Case of Subconjunctival Dirofilariasis by Dirofilaria Repens in Rural Gujarat. *Indian Journal of Ophthalmology* **62**: 649-51.

Reddy, Maryada Venkatarami. (2013). Human Dirofilariasis: An Emerging Zoonosis. *Tropical Parasitology* **31**: 2-3.

Sangit, V.A. & Haldipurkar, S.S. (2012). Subconjunctival Dirofilariasis Mimicking Scleritis: First Report from Western India. *Indian Journal of Ophthalmology* **60**: 76-77.

Sathyan, P., Manikandan, P., Bhaskar, M., Padma, S., Singh, G. & Appalaraju, B. (2006). Subcutaneus Infection by Dirofilaria Repens. *Indian Journal of Medical Microbiololy* **24**: 61-62.

Simón, F., Siles-Lucas, M., Morchón, R., González-Miguel, J., Mellado, I., Carretón, E., Montoya-Alonso, J.A. (2012). Human and Animal Dirofilariasis: The Emergence of a Zoonotic Mosaic. *Clinical Microbiology Review* **25**: 507-44.

Simsek, Sami & Ayse Turkan Ciftci. (2016). Serological and Molecular Detection of Dirofilaria Species in Stray Dogs and Investigation of Wolbachia DNA by PCR in Turkey. *Journal of Arthropod - Borne Diseases* **10**: 445-53.

Sukpanichnant, S.I., Leenuttapong, V., Dejsomritrutai, W., Thakerngpol, K., Wanachiwanawin, W., Kachintorn, U. & Nitiyanant, W. (1998). Pulmonary Dirofilariasis in a Patient with Multisystem Langerhans Cell Histiocytosis – The First Reported Case in Thailand. *Journal of the Medical Association of Thailand* **81**: 722-27.

Tsung, S.H. & Liu, C.C. (2003). Human Pulmonary Dirofilariasis in Taiwan. *Journal of the Formosan Medical Association* **102**: 42-45.

Wong, Ming M. & Brummer, M.E.G. (1978). Cuticular Morphology of Five Species of Dirofilaria: A Scanning Electron Microscope Study. *The Journal of Parasitology* **64**: 108-14.

Yilmaz, E., Fritzenwanker, M., Pantchev, N., Lendner, M., Wongkamchai, S., Otranto, D., Kroidl, I., Dennebaum, M., Le, T.H., Anh Le, T., Ramünke, S., Schaper, R., von Samson-Himmelstjerna, G., Poppert, S. & Krücken, J. (2016). The Mitochondrial Genomes of the Zoonotic Canine Filarial Parasites Dirofilaria (Nochtiella) Repens and Candidatus Dirofilaria (Nochtiella) Honkongensis Provide Evidence for Presence of Cryptic Species. *PLoS Neglected Tropical Diseases* **10**: 1-22.