Dirofilariasis in Vietnam: A case report and brief review

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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 Dirofilaria 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

<table>
<thead>
<tr>
<th>N</th>
<th>Gender</th>
<th>Age</th>
<th>Location</th>
<th>Agent</th>
<th>Residence</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>30</td>
<td>Eyelid</td>
<td>D. repens</td>
<td>Ha Noi</td>
<td>2008</td>
<td>Dang et al., 2010</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>50</td>
<td>Sub-conjunctiva</td>
<td>D. repens</td>
<td>Ha Noi</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>47</td>
<td>Sub-conjunctiva</td>
<td>D. repens</td>
<td>Ha Noi</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
<td>4</td>
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<td>27</td>
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<td>D. repens</td>
<td>Ha Noi</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
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<td>D. repens</td>
<td>Ha Noi</td>
<td>2006–2010</td>
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</tr>
<tr>
<td>6</td>
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<td>77</td>
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<td>D. repens</td>
<td>Ninh Binh</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
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<td>2006–2010</td>
<td>De et al., 2012</td>
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<td>D. repens</td>
<td>Hung Yen</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
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<td>D. repens</td>
<td>Hung Yen</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
<td>10</td>
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<td>Ha Nam</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
</tr>
<tr>
<td>11</td>
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<td>Ha Nam</td>
<td>2006–2010</td>
<td>De et al., 2012</td>
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<tr>
<td>12</td>
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<td>D. repens</td>
<td>Ha Noi</td>
<td>2011</td>
<td>Le et al., 2015</td>
</tr>
<tr>
<td>13</td>
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<td>64</td>
<td>Sub-cutaneous</td>
<td>D. repens</td>
<td>Ha Noi</td>
<td>2018</td>
<td>This case</td>
</tr>
</tbody>
</table>

(477) The 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 dH2O 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 fact, 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 Malarialogy, 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), peri-orbital 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-nucaosa 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* (Sathyen 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 16S RNA 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 dirofilariasis 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.

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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**


