

Onchocerca lupi infection in Turkey: A unique case of a rare human parasite

Hatice Deniz Ilhan^{1*}, Aylin Yaman², Yasuyuki Morishima³, Hiromu Sugiyama³, Maki Muto³,
Hiroshi Yamasaki³, Hideo Hasegawa⁴, Banu Lebe⁵ and Meltem Soylev Bajin²

¹Department of Ophthalmology, Akdeniz University, Antalya, Turkey; ²Department of Ophthalmology, Dokuz Eylul University, Izmir, Turkey; ³Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan; ⁴Department of Biology, Oita University, Oita, Japan; ⁵Department of Pathology, Dokuz Eylul University, Izmir, Turkey

Abstract

Onchocerca lupi was first isolated from a wolf in Russia. Since then, canine ocular onchocercosis has been increasingly reported, particularly in Europe and the United States. It is thought that blackflies and midges are the vectors of transmission, and it is possible that these vectors could transmit the parasite to humans. The first human case of *O. lupi* in Turkey was reported in 2011. In this report we present the third human case of *O. lupi* infection in Turkey. Our patient was a 28-year-old male who displayed a painless, immobile mass under the conjunctiva. The mass measured 10 x 12 mm in size. Pathological examination of the surgically excised tissue was suggestive of infection by a filarial nematode. Subsequently, the parasite was identified as *O. lupi* through molecular analysis. All of the previously reported cases of *O. lupi* in both humans and dogs were more symptomatic than in our patient, *Onchocerca* infection should not be ruled out during the differential diagnosis of the subconjunctival and orbital cystic mass in instances where there is little to no inflammation. It is important to consider biopsy and carry out molecular analysis to identify the parasite.

Keywords

Onchocerca lupi, orbita, Turkey, zoonotic infestation

Introduction

Ocular onchocercosis is a zoonotic parasitic disease caused by filarial nematodes of the genus *Onchocerca*. One of the species within this genus is *Onchocerca lupi*, which is known to parasitize dogs and other canids. *O. lupi* was first described from a sample taken from a wolf in Russia (Rodonaja 1967). Since then, canine ocular onchocercosis has increasingly been reported, particularly in Europe and the United States (Sréter and Széll 2008). Canine onchocercosis has been reported as both acute and chronic ocular and periocular infections, with either eye susceptible to infection. Additionally, a wide variety of ophthalmic manifestations have been reported, ranging from conjunctivitis to exophthalmos (Sréter and Széll 2008). It is thought that blackflies and midges are the vectors of transmission, and it is possible that these vectors could transmit the parasite to humans. The first two verified cases of *O. lupi* parasitization in humans occurred in the northwest of Turkey and

were described by Otranto *et al.* (2011, 2012). In this report we describe the third confirmed human case of an *O. lupi* infection in Turkey.

Materials and Methods

A 28-year-old Turkish male with a tangible mass under the bulbar conjunctiva in the right eye was admitted to the Department of Ophthalmology at Dokuz Eylul University in Izmir, Turkey. The individual worked as a farmer in a village near Izmir (38°25'N, 27°09'E), a city on the Turkish Aegean coast. He had never traveled abroad nor in other regions of Turkey. Furthermore, there was no evidence of an insect bite nor was there a history of an animal attack.

Upon ophthalmologic examination, a painless, immobile mass measuring approximately 10 x 12 mm in size was detected in the patient's right eye. The patient's right eye was also slightly pseudoptotic (Fig. 1a). The anterior margin of the mass

*Corresponding author: drdenizilhan@gmail.com

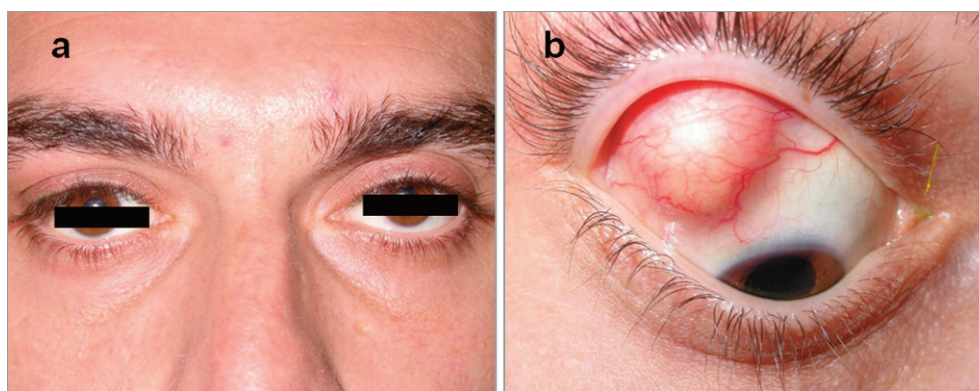


Fig. 1. Ophthalmologic findings. **a.** Right eye is slightly pseudoptotic; **b.** Mild lid edema and mild conjunctival hyperemia around the mass were seen

was superotemporally located 5 mm away from the limbus. The mass also extended posteriorly through the orbital septum, near the superior rectus muscle. There was also a small amount of lid edema and conjunctival hyperemia around the mass. The patient's right eye was observed in a downward gaze (Fig. 1b). The patient had a corrected visual acuity of 20/20. In addition, the anterior and posterior segments as well as the intraocular pressure were all found to be normal in both eyes.

Orbital magnetic resonance imaging (MRI) was also used to properly discern the structure of the mass (Fig. 2). Both the signal and volume enlargement of the anterosuperolateral part of the right orbita were observed. Additionally, there was cystic formation between the sclera and the conjunctiva, with enlargement of the surrounding tissue. There were no other systemic findings.

A surgical excision of the mass was performed. During the surgery the semicystic mass was perforated and the parasite within was cut accidentally. The parasite was then excised and

processed for histopathological examination. In order to identify the parasite to species at the DNA level, the formalin-fixed and paraffin-embedded (FFPE) specimen was sent to the Department of Parasitology at the National Institute of Infectious Diseases in Tokyo, Japan.

The patient returned 10 months later for a follow-up visit, and both the biomicroscopic and ophthalmoscopic examinations were normal. Furthermore, no parasite was found in the anterior chamber or in the vitreous cavity nor were there any other symptoms associated with the past infection.

DNA from the FFPE specimen was prepared using a DEX-PAT® (Takara Bio, Japan), according to the manufacturer's instructions. For the molecular identification of the parasite, the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 5 (*nad5*) genes were amplified by polymerase chain reaction (PCR). The primers used were newly designated to be amplified short-size products (up to 250 bp) based on these genes of the filarial nematodes (*Onchocerca* and *Dirofilaria* spp.). The primers used were 5'-GCTTTGTCTTTTGGTTTACTTTTG-3' and 5'-GTTGGG TGCTATTAATTTTATGG-3' for *cox1*, and 5'-CCTGTTA GTTGTGTTGGTTCATAGTAG-3' and 5'-GGTTTTGTTTT TTGGCTATTGGTAG-3' for *nad5*. PCR amplification was carried out in a total volume of 30 µL of reaction mixture with *ExTaq* DNA polymerase (Takara Bio, Japan) and performed for 30 cycles of denaturation (98°C, 30 sec), annealing (54°C, 30 sec) and extension (72°C, 30 sec), plus one cycle of 72°C for 5 min. DNA sequencing was performed according to the method previously reported (Dang *et al.* 2010). Molecular identification was performed based on the genetic distance values and phylogenetic analysis of the *cox1* and *nad5* sequences.

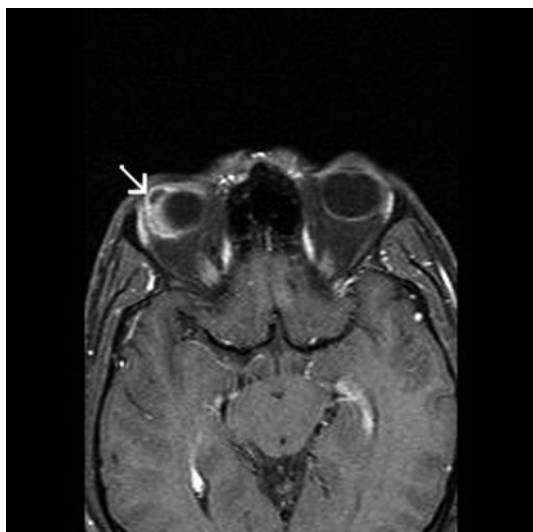


Fig. 2. Orbital MRI findings of the patient. White arrow showed the lesion. Note a cystic formation between sclera and conjunctiva with enlargement of right orbita

Results

Morphological features of the parasite

Although the parasite was partially damaged (it had been accidentally cut during the surgical removal of the mass and its



Fig. 3. Histopathological findings of the parasite. Arrows indicates prominent annular ridges on the external surface and transverse striae in the underlying layer are indicated by arrowheads. C, cuticle; U, uterus; I, intestine. Hematoxylin-eosin stain

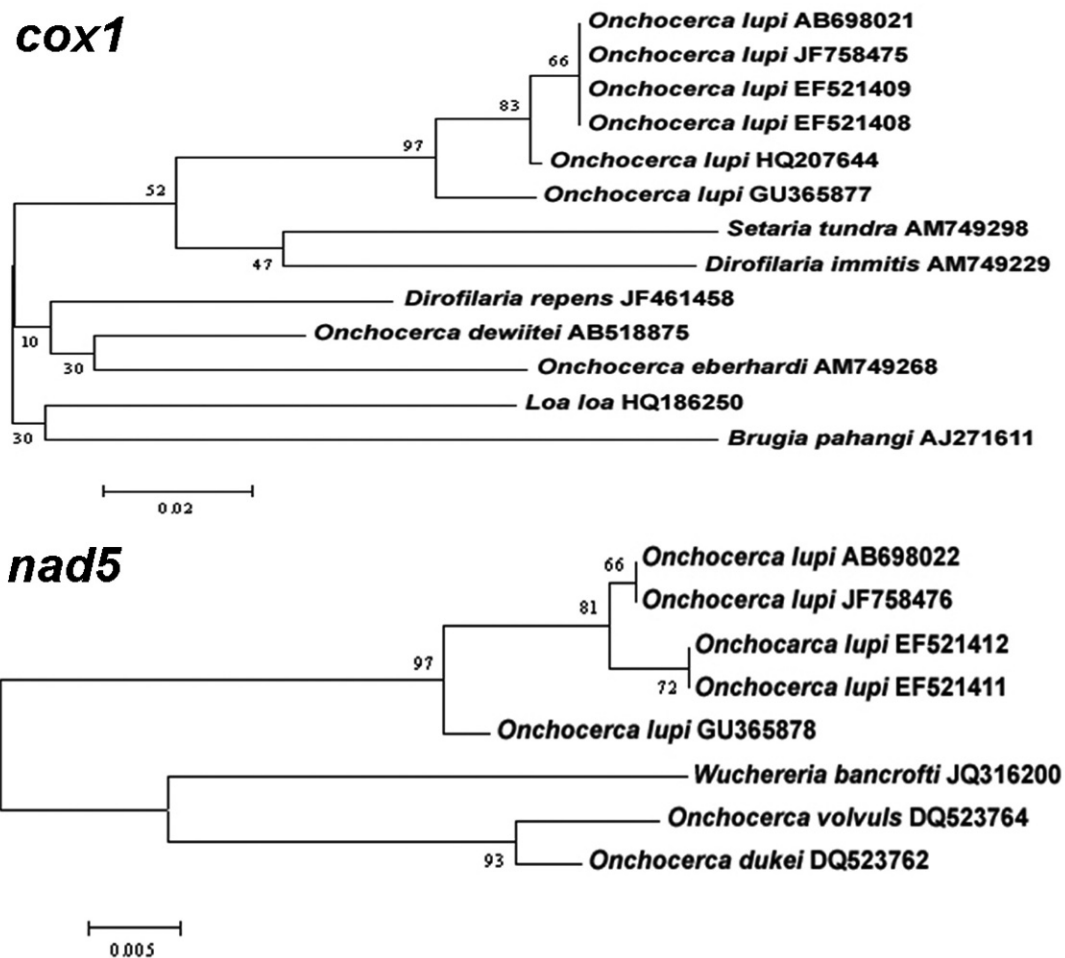


Fig. 4. Phylogenetic analysis of *Onchocerca* and other filarial nematodes inferred from the *cox1* (above) and *nad5* (below) genes by neighbor-joining method

internal organs had collapsed), some morphological features were able to be discerned (Fig. 3). The cross-section of the parasite showed a thick, multilayered cuticle ($> 8\mu\text{m}$) which had prominent annular ridges on the external surface (interval from 36.5–44.5 μm , arrows). Also, in the underlying layers of the cuticle transverse striae were observed (arrowheads in Fig. 3). The number of striae between consecutive annular ridges is useful in identifying *Onchocerca* species (Orihel *et al.* 1991 and Eberhard *et al.* 2000), and in this specimen there were two striae. In addition, reproductive organs with a thick wall were observed, strongly suggesting that this parasite was a female filarioid belonging to the genus *Onchocerca*.

Molecular analysis

Cox1 and *nad5* gene fragments were successfully amplified using PCR (data not shown). A comparison of the nucleotide sequences of *cox1* genes from our *Onchocerca* sp. specimen (AB698021) and *O. lupi* isolates from Europe found that the genetic distance values between the samples were low ($d = 0.000\text{--}0.018$), indicating typical intraspecific variation. A comparison of the *nad5* genes from our *Onchocerca* sp. (AB698022) and *O. lupi* isolates from Europe and the United States were also at values that correspond to intraspecific variation ($d = 0.000\text{--}0.056$). The filarial parasite was thus identified as *Onchocerca lupi*.

Discussion

The majority of infections caused by *O. lupi* were reported as ocular diseases in canines. It is thought that canines are the main hosts of *O. lupi* (Sréter and Széll 2008). It could therefore be the case that human infections are acquired accidentally, by some means.

The present study reports the third confirmed case of *O. lupi* in a human in Turkey, as confirmed by molecular analysis. Interestingly, there are no reports of *O. lupi* infections in dogs in Turkey. The patient reported in this study was from a different geographical area than the first two cases reported by Otranto *et al.* (2011, 2012). While the first two cases occurred in the northwest of Turkey, our patient came from southwest, about 550 km away from the previous two cases. The details of confirmed human cases caused by *Onchocerca lupi* are summarized in Table I.

The other four ocular *Onchocerca* infections were diagnosed based on the morphology of the parasite.

Pampiglione *et al.* (2001) presented a subconjunctival infection caused by *Onchocerca* species, with the parasite being identified based on morphology (Pampiglione *et al.* 2001). Additional subconjunctival cases were verified by Otranto *et al.* using molecular analysis (Otranto 2011, 2012). In each of these subconjunctival infections, the patient had congestion, pain and inflammation. Our patient, however, had neither pain nor discomfort. Furthermore, although the cystic lesion was fixed to the sclera, there was no penetration. In the instance of Pampiglione *et al.*, for example, the parasitic mass penetrated both the sclera and the iris of the patient (Pampiglione *et al.* 2001). Sallo *et al.* (2005) reported a parasite in the vitreous cavity and Burr *et al.* (1998) reported one found in the cornea (Sallo *et al.* 2005 and Burr *et al.* 1998). Additionally, the first case presented by Azarova *et al.* (1965) was in the extraocular muscle tendon.

Although all of the previous cases reported in both humans and dogs were more symptomatic, our patient showed only mild inflammation. Therefore, *Onchocerca* infection should not be ruled out during the differential diagnosis of the subconjunctival and orbital cystic mass in instances where there is little to no inflammation.

Based on its scarcity in the literature, it would appear as though infection by *O. lupi* is rare in humans in general. However, filarial infections of the human eye are not uncommon. Often with these filarial infections, the individual parasite is not even identified to species level. In the present case, molecular analysis using a FFPE specimen was necessary to achieve a definitive identification of the parasite. All of this may suggest that other nematodes which infest the human eye may often be either under-identified (i.e. only to generic not specific level) or misidentified, thus obscuring the real frequency of *O. lupi* infections. It is therefore recommended that the parasite be identified to species level using molecular analysis whenever possible.

Furthermore, in order to better understand the life cycle of *O. lupi* and learn more about its threat to humans, additional research needs to be conducted to identify its reservoirs and vectors and its transmission patterns.

Acknowledgements. This work was supported in part by a Grant from Akdeniz University Scientific Projects Unit to HDI, and Grants-

Table I. Confirmed onchocercosis *lupi* in humans

Case#	Country	Age	Sex	Morphological identification	Molecular identification	Reference
1	Turkey	18	Female	<i>O. lupi</i>	<i>O. lupi</i>	Otranto <i>et al.</i> 2011
2	Turkey	26	Male	<i>O. lupi</i>	<i>O. lupi</i>	Otranto <i>et al.</i> 2012
3	Tunisia	8	NA†	<i>O. lupi</i>	ND‡	Otranto <i>et al.</i> 2012
4	Turkey	28	Male	<i>O. lupi</i>	<i>O. lupi</i>	The present case

† NA, not available

‡ ND, not determined

in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare, Japan (H20-Shinko-Ippan-016) to YM and HY.

References

- Azarova. N.S., Miretskii O.I., Sonin M.D. 1965. The first instance of detection of nematode *Onchocerca* Diesing, 1841 in a person in the USSR. *Medical Parazitology*, 34, 156–158 (in Russian).
- Burr W.E. Jr., Brown M.F., Eberhard M.L. 1998. Zoonotic *Onchocerca* (Nematoda: Filarioidea) in the cornea of a Colorado resident. *Ophthalmology*, 105(8), 1494–1497.
- Dang T.C.T., 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, 1003–1007.
- Eberhard M.L., Ortega Y., Dial S., Schiller C.A., Sears A.W., Greiner E. 2000. Ocular *Onchocerca* infections in western United States. *Veterinary Parasitology*, 90, 333–338.
- Orihel T.C., Ash L.R., Holshuh H.J., Santenelli S. 1991. Onchocerciasis in a California dog. *American Journal of Tropical Medicine and Hygiene*, 44, 513–517.
- Otranto D., Sakru N., Testini G., Gürlü V.P., Yakar K., Lia R.P., Dantas-Torres F., Bain O. 2011. Case report: First evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae). *American Journal of Tropical Medicine and Hygiene*, 84, 55–58. DOI: 10.4269/ajtmh.2011.10-0465.
- Otranto D., Dantas-Torres F., Cebeci Z., Yeniad B., Buyukbabani N., Boral O.B., Gustinelli A., Mounir T., Mutafovchiev Y., Bain O. 2012. Human ocular onchocerciasis: further evidence on the zoonotic role of *Onchocerca lupi*. *Parasites & Vectors*, 5, 84. DOI: 10.1186/1756-3305-5-84.
- Pampiglione S., Vakalis N., Lyssimachou A., Kouppari G., Orihel T.C. 2001. Subconjunctival zoonotic *Onchocerca* in an Albanian man. *Annals of Tropical Medicine & Parasitology*, 95, 827–832.
- Rodonaja T.E. 1967. A new species of nematode, *Onchocerca lupi* n. sp., from *Canis lupus cubanensis*. *Soobshcheniya Akademii Nauk Gruzinskoy SSR*, 45, 715–719 (in Russian).
- Sallo F., Eberhard M.L., Fok E., Baska F., Hatvani I. 2005. Zoonotic intravitreal *Onchocerca* in Hungary. *Ophthalmology*, 112, 502–504.
- Sréter T., Széll Z. 2008. Onchocercosis: a newly recognized disease in dogs. *Veterinary Parasitology*, 151, 1–13.

(Accepted June 10, 2013)