Dirofilaria Repens as a Cause of Subconjunctival Infection in a 77-Years Old Female Patient from Croatia – A Case Report

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A B S T R A C T

The zoonotic parasites of the genus Dirofilaria are on the increase as an accidental finding or as a cause of disease in humans worldwide. Human dirofilariasis usually manifests as either subcutaneous infiltrates or lung parenchymal disease, in many cases asymptomatically. We report the case of a 77-year old female patient presenting with irritation and pain in her left eye. Ophthalmologic examination of the temporal part of her left eye revealed motile and threadlike organism, which was surgically extracted and morphologically identified as Dirofilaria. Further molecular diagnostics with polymerase chain reaction (PCR) confirmed that the isolated organism is Dirofilaria repens. Due to already recognized autochthonous occurrence of human dirofilariasis in Croatia, human dirofilariasis must be included in the differential diagnosis of patients presenting with subcutaneous nodules, eye affection and other potential manifestations of this disease.

Key words: *dirofilariasis, nematode, subconjunctival infection, ophthalmology, parasitology*

Introduction

Representatives of the nematode, genus *Dirofilaria*, particularly *Dirofilaria immitis*, *Dirofilaria repens*, *Dirofilaria tenuis* and *Dirofilaria ursi*, are becoming a common finding either as an accidental discovery or during diagnostic assessment of patients around the world presenting with certain symptoms[1]. Dirofilariae are natural parasites of a great variety of animals and, with the exception of *D. immitis*, they live in the subcutaneous tissue of their definitive host producing circulating microfilariae which can be transmitted by various arthropods.

Humans are accidental hosts for *Dirofilaria* genus with patient symptomatic infections being extremely rare. Most documented human cases of infections with this genus are attributed to *D. repens*. As domestic and wild canids are definitive hosts of *D. repens*, the dog is the principal reservoir for infection with this species. Mosquitoes of the genus *Aedes*, *Anopheles* and *Culex* serve as intermediate hosts and vectors, and some species of ticks, lice and flies are suggested as possible vectors as well[1,2].

D. repens infection in humans is usually manifested as skin or subconjunctival infiltrates. These nematodes do not normally reach sexual maturity in the human organism. Consequently microfilaremia is not expected to develop in humans. However, one case of subcutaneous *D. repens* infection with microfilariae in the circulation has been described in the literature[3].

Clinical importance of this infection lies in the fact that dirofilariasis in humans is commonly misdiagnosed as tumour or some other granulomatous disease, which can lead to unnecessary and often invasive diagnostic tests and procedures. It was decided to present in this short report a case of a female patient with conjunctival form of this disease.

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Case Report

A 77-year-old female patient was admitted to the ophthalmology ward of General Hospital dr. Ivo Pedišić in Sisak for intermittent sensation of scratching, rubbing and sharp, thrusting pain in her left eye. She also experienced a sensation of movement in her eye, which later became visible.

Medical history revealed that the patient is in charge of a large rural estate with a lot of dogs and cats present. An epidemiologically important detail was frequent previous mosquito bites, through the patient had never travelled or resided outside Croatia.

Ophthalmologic examination of the temporal part of her left eyeball revealed a motile, threadlike structure. Biomicroscopy of the eye performed temporally presented a long and thin live parasite under the chemotic and injected bulbar conjunctiva, showing occasional serpentine movement. Light stimulus applied to the eye elicited response from the organism in the sense of squatting and slowly calming down. Cornea was clear, and anterior eye chamber had normal content and depth. Further eye findings were normal in accordance with age of the patient. After clinical suspicion of dirofilariasis was established, operation was indicated. Upon surgical extirpation of the parasite under local anaesthesia, the motile larva about 10 centimetres (4 inches) in length was placed in a sterile test tube containing saline solution and sent to the Parasitology Department of the Croatian National Institute of Public Health in Zagreb. On the basis of morphologic characteristics, it was confirmed that the extirpated larva belongs to the *Dirofilaria* genus. Polymerase chain reaction (PCR) confirmed that the organism is *Dirofilaria repens*.

Postoperative ultrasound of the patient's left eye showed normal findings.

Materials and Methods

Extraction of the parasitic DNA was done using commercial Blood and Tissue Kit (QIAGEN) system, according to manufacturer's instructions. Molecular identification of the parasite was conducted using DIDR-F1 (5' – AGT GCG AAT TGC AGA CGC ATT GAG – 3') and DIDR-R1 (5' – AGC GGG TAA TCA CGA CTG AGT TGA – 3') primers. Reaction mixture consisted of 1 x PCR buffer, 1.5 mM $MgCl_2$, 10 pmol of each primer, 200 mM dNTP and 1.5 U GoTaq polymerase (Promega). Amplification was carried out using a method developed by Rinshiw and co-authors[4]. Primers were used to amplify Internal transcribed spacer region 2 (ITS2) for *D. immitis* (542 bp), *D. reconditum* (578 bp) and *D. repens* (484 bp) on Real Time PCR, Applied Biosystems Veriti machine.

Discussion

Human infection with *Dirofilaria* can be manifested in various forms. The majority of infestations are caused by *D. repens* species, which usually leads to development of subcutaneous nodules anywhere in the body, with a possibility of eye invasion[5].

Dirofilariasis is an endemic parasitic disease in the Mediterranean Basin and territories of Ukraine and south Russia[6]. Review of literature on this topic suggest that Italy is a European country with the highest prevalence of human dirofilariasis, followed by France, Greece and Spain[5,7,8]. Global warming and hot summers are important contributing factors to the spread of *Dirofilaria*. Consequently, this parasite can be expected to appear in previously unaffected areas of Europe[9]. The first human case of autochthonous *D. repens* infection in Hungary was described in 2008[10]. Autochthonous occurrence of human dirofilariasis has been confirmed in Ukraine and Russia as well, with more and more diagnosed cases every year[11,12]. As an endemic organism, this parasite has been found in the territories of Asia Minor, central Asia and Sri Lanka[13].

About ten cases of human dirofilariasis have been described in Croatia to date, with nematode *D. repens* confirmed as the sole causative agent. In the first described case from the year 1996, the affected region was the eye and conjunctiva[14], while in 2003, the first cases of subcutaneous form of human dirofilariasis in Croatia were published[15]. From the clinical point of view, one of the most interesting cases was an infection manifested as subcutaneous, motile and painless nodule located in the left breast of a 52-year-old woman, initially suggestive of a malignant process[16]. All these examples of human subcutaneous dirofilariasis were described in patients from the Mediterranean part of Croatia[17]. In 2007, a rare case of lacrimal gland dirofilariasis from inland Croatia was described, the second of its kind in the literature[18]. The patient's general and ophthalmic condition was good, and infection clinically manifested as disfiguring swelling in the lateral part of the left upper eyelid, without any pain or loss of vision. Two more cases of subcutaneous dirofilariasis were described in the same year, in patients who neither travelled to endemic parts of Croatia nor abroad[2]. Recently, one new case of ocular dirofilariasis in Croatia has been published, clinically similar to our report[19].

Key elements for accurate diagnosis of human dirofilariasis are clinical presentation of infection, as well as macroscopic and histopathologic examinations of bioptic material. Due to unspecific results, serologic diagnostics of dirofilariasis is not routinely used[20], while PCR is typically applied as the confirmatory test. The number of reported cases of human dirofilariasis and affected countries has increased in the last decades, which is supported by a growing number of studies and case reports. Several contributing factors might be responsible, including changes in people's lifestyle, greater medical awareness in larger number of countries, increased travel to endemic areas, global warming and climatic changes that facilitate the introduction of competent vectors into new geographic areas, as well as a lack of adequate control measures in animal reservoirs[21].

Albeit rare, both clinicians and parasitologists should consider and include dirofilariasis in the differential diagnosis of patients presenting with subcutaneous nodules, eye affection and other potential manifestations of this disease.

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DIROFILARIA REPENS KAO UZROČNIK SUBKONJUNKTIVALNE INFEKCIJE U 77-GODIŠNJE PACIJENTICE IZ HRVATSKE – PRIKAZ SLUČAJA

SAŽETAK

Zoonotski paraziti iz roda Dirofilaria u porastu su kao slučajan nalaz ili kao uzrok bolesti u ljudi diljem svijeta. Ljudska dirofilarijaza obično se manifestira u vidu potkožnih čvorića ili kao bolest plućnog parenhima, u velikom broju slučajeva potpuno asimptomatski. Prikazujemo slučaj 77-godišnje pacijentice s pojavom iritacije i boli u lijevom oku. Oftalmološkim pregledom sljepoočnog dijela njenog lijevog oka otkriven je pokretni organizam nalik končiću, koji je kirurški odstranjen i morfološki identificiran kao parazit iz roda Dirofilaria. Daljnja molekularna dijagnostika lančanom reakcijom polimeraze (PCR) potvrdila je da se radi o vrsti Dirofilaria repens. Zbog već opisane autohtone ljudske dirofilarijaze u Hrvatskoj, dirofilarijaza se mora diferencijalno-dijagnostički razmotriti kod pacijenata s potkožnim čvorićima, zahvaćanjem oka i drugim potencijalnim manifestacijama ove bolesti.