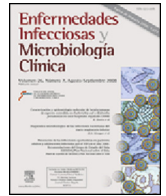




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Original article

Thirty cases of human subcutaneous dirofilariasis reported in Rostov-on-Don (Southwestern Russian Federation)



Boris Ilyasov^a, Vladimir Kartashev^b, Nikolay Batrikov^c, Liudmila Madjugina^d, Javier González-Miguel^e, Rodrigo Morchón^e, Fernando Simón^{e,*}

^a Department of Diagnostic Ultrasound, Rostov Oblast Diagnostic Centre, Rostov-na-Donu, Russia

^b Department of Infectious Diseases, Rostov State Medical University, Rostov-na-Donu, Russia

^c Department of Ophthalmology, Hospital of the North Caucasus Branch of the Russian Railway, Rostov-na-Donu, Russia

^d Department of Diagnostic Ultrasound, Hospital of the North Caucasus Branch of the Russian Railway, Rostov-na-Donu, Russia

^e Laboratory of Parasitology, Faculty of Pharmacy and Institute of Biomedical Research of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain

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ABSTRACT

Introduction: Different species of the genus *Dirofilaria*, mainly *D. immitis* and *D. repens*, are responsible for emergent vector borne transmitted zoonotic diseases in the Old World. Human *D. repens* infections are characterized by the appearance of benign subcutaneous nodules that mimic skin malignant tumors or due to live worms in the ocular area.

Methods: Thirty patients presenting superficial or deep nodules were treated at the Rostov Regional Diagnostic Center (Southwestern Russian Federation). Anatomical characteristics of the nodules were studied by non-invasive ultrasound and color and power Doppler techniques. Worms were surgically removed from every nodule and their DNA analyzed by polymerase chain reaction (PCR).

Results: Twenty-four out of the 30 nodules were located in the hypodermis, two in a retro-ocular location and four in scrotal location. Image techniques allowed the identification of the helminthic origin of all nodules, based on their oval and regular shape, peripheral vasculature, and the existence of internal linear winding hyperechoic structures with or without movements, indicating the presence of live or dead worms, respectively. Specific adscription to *D. repens* was achieved by the PCR analysis.

Conclusion: The series described in the present paper confirms the increasing risk of infection by *D. repens* in humans living in endemic areas of Eastern Europe. The use of non-invasive ultrasounds and Doppler techniques can contribute to an appropriate management of human dirofilariasis. Moreover, awareness of the medical community in the endemic areas influences the report of cases, and consequently the current epidemiological picture of human dirofilariasis.

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Treinta casos de dirofilariasis subcutánea humana reportados en Rostov del Don (suroeste de la Federación Rusa)

RESUMEN

Introducción: Diferentes especies del género *Dirofilaria*, principalmente *D. immitis* y *D. repens*, son responsables de enfermedades zoonóticas transmitidas por vectores en el viejo mundo. Las infecciones humanas causadas por *D. repens* se caracterizan por la aparición de nódulos subcutáneos benignos que se confunden con tumores malignos de piel o por la localización de vermes vivos en la zona ocular.

Métodos: Treinta pacientes con nódulos superficiales o profundos fueron tratados en el Centro de Diagnóstico Regional de Rostov (suroeste de la Federación Rusa). Las características anatómicas de los nódulos fueron estudiadas mediante ultrasonidos no invasivos y Doppler de color y potencia. Los vermes se retiraron quirúrgicamente de cada nódulo y su ADN se analizó mediante la reacción en cadena de la polimerasa (PCR).

Palabras clave:

Dirofilaria repens

Dirofilariasis subcutánea/ocular

Ultrasonidos

Doppler

Reacción en cadena de la polimerasa

* Corresponding author.

E-mail address: fersimon@usal.es (F. Simón).

Resultados: Veinticuatro de los 30 nódulos se localizaron en la hipodermis, 2 en localización retroocular y 4 en la zona escrotal. Las técnicas de imagen permitieron la identificación del origen helmíntico de todos los nódulos, en base a su forma ovalada y regular, la vasculatura periférica y la existencia de estructuras lineales internas hiperecoicas con o sin movimientos, indicando la presencia de gusanos vivos o muertos, respectivamente. La adscripción específica a *D. repens* se logró mediante el análisis de PCR.

Conclusión: La serie de casos descrita en el presente trabajo confirma el creciente riesgo de infección por *D. repens* en las personas que viven en zonas endémicas del Este de Europa. El uso de técnicas no invasivas como los ultrasonidos y el Doppler puede contribuir a un adecuado manejo de la dirofilariosis humana. Por otra parte, el conocimiento de la enfermedad por parte de la comunidad médica de las áreas endémicas influye en el informe de los casos y, consecuentemente, en el cuadro epidemiológico actual de la dirofilariosis humana.

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Introduction

Zoonotic filarioses are vector borne transmitted diseases caused by parasitic filarial nematodes of the genus *Dirofilaria*, mainly *D. immitis* and *D. repens*. These two species are primarily parasites of domestic and wild canines and felines all over the world¹ being transmitted by different species of culicid mosquitoes belonging the genera *Culex*, *Aedes* and *Anopheles*.² Because many of these species feed indiscriminately on animal reservoirs and man, in areas where canine dirofilariosis is endemic, the risk of human infections exists.³ In Europe, canine dirofilariosis caused by *D. repens* is spreading from the traditionally endemic Southern Mediterranean countries to the central and Northern countries, being currently considered an emerging disease in these areas. In parallel, a dramatic increase of human subcutaneous/ocular dirofilariosis is being detected, with more than 2850 cases reported until now in the world literature, many of them diagnosed in the last 13 years in Ukraine and Southwestern Russia.^{4,5} These changes are attributed to the global warming, human interventions modifying environment and pets management.⁶ Human subcutaneous dirofilariosis is a condition characterized by the development of subcutaneous nodules caused by the local inflammatory reaction against *D. repens* worms, usually immature, that mimic both benign and malignant primary or metastatic skin tumors.⁷ In addition live, fully developed adult worms can lodge in intraocular and retroocular locations, causing considerable damages and discomfort in patients by themselves or as a consequence of their surgical removal.^{8,9} Diagnosis of human subcutaneous dirofilariosis raises important procedure problems because of the initial suspicion of a malignant origin of the nodules and the very frequent absence of parasite larvae (microfilariae) in the blood of patients.⁴ Thus, most cases are diagnosed by histology and morphological and/or molecular analysis, subsequent to the surgical removal of the whole cysts or the worm themselves.^{7,10,11}

The aim of this work is to report an extensive series of human subcutaneous/ocular dirofilariosis cases observed during an 11-year period (2003–2013) in the Rostov Oblast Diagnostic Center, located in the hyperendemic area of canine dirofilariosis in Southwestern Russian Federation.

Methods

Cases of human subcutaneous dirofilariosis were retrospectively collected from the case record of the Rostov Regional Diagnostic Center Department of Ultrasound. This center functions as referral medical center of the Rostov region which has a population of 4,277,976 inhabitants. Cases were referred from ophthalmologists, surgeons, oncologists, urologists, or from other physicians for assessment of subcutaneous or more deeply located nodules.

The data were gathered reviewing the clinical charts or the clinical, laboratory and imaging registries.

Ultrasound examinations were performed in all patients on ultrasound equipment SonoCT HDI-5000 and iU22 “Philips” (Philips Healthcare, Bothell, WA, USA), using a broadband linear probes with an aperture of 38 mm and the frequency of 5–12 MHz and 5–17 MHz. Patients were examined with multiple views scanning with the special program for superficial tissues study in B-mode as well as using a regimen of color and power Doppler with spectral curve analysis of blood flow. Ultrasounds study evaluated the skin thickness, the echogenicity and echo structure of the nodule itself and the structure of surrounding superficial and deeper tissues. Every nodule was characterized by the size, shape, borders, homogeneity, detailed characteristics of internal echo structure, presence or absence of blood flow and vascular pattern. All the nodules were afterwards surgically excised and the removed worms analyzed by the PCR technique with specific primers as described by Favia et al.¹² In brief, DNA was extracted from each parasite with the DNeasy Blood & Tissue Kit (Qiagen, GmbH, D-40724). After activation of the Taq Gold polymerase at 94 °C for 12 min, PCR reactions were performed as follows: 94 °C (30 s), 65 °C (30 s), 72 °C (30 s) for 35 cycles and a final step at 72 °C for 7 min. PCR products were visualized on agarose gel.

All patients provided informed consent to participate in this study having been approved by the Ethical Committee of the Rostov State Medical University.

Results

Between 2003 and 2011, 30 patients (21 females) were diagnosed of dirofilariosis. Patients noticed a single superficial or deep nodule without history of previously diagnosed malignancies. Data on the patients, characteristics and location of the nodules appear in Table 1. The most common presentation was a painless subcutaneous nodule, visible with naked eyes (26/30) or found by palpation (2/30). Twenty-four out of the 30 nodules were located in the hypodermis and the other six were located in deeper locations. From these, two out of the 10 ocular cases appeared in retroocular location, and four additional cases in the scrotum. All the patients with ocular dirofilariosis reported history of swelling of the lower or upper eyelids or periorbital edema, burning sensation, itching, painful eye turns, conjunctival hyperemia and tearing. Some of them had the feeling of a foreign body in the eye. The patients with scrotal lesions presented with non-tender local soft tissue mass located separately from testicular appendages. One of the patients noticed a gradual increasing edema as well as hyperemia of one half of the scrotum without definite mass lesion. In other patient physical examination revealed a soft painless mass closely adjacent to the caudal portion of the epididymis (the first clinical impression was “a cyst of caudal epididymis”). Patients with other location of

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