Original article

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

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A B S T R A C T

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 ultrasound 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 DIROFILOARIOSIS SUBCUTÁNEA HUMANA REPORTADOS EN ROSTOV DEL DON (Suroeste de la Federación Rusa)

R E S U M E N

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 en función de seres 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 seres vivos se retiraron quirúrgicamente de cada nódulo y su ADN se analizó mediante la reacción en cadena de la polimerasa (PCR).

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Palabras clave:
Dirofilaria repens
Dirofilariosis subcutánea/ocular
Ultrasónidos
Doppler
Reacción en cadena de la polimerasa

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Resultados: Veinticuatro de los 30 nódulos se localizaron en la hipodermitis, 2 en localización retroocular y 4 en la zona escostral. Las técnicas de imagen permitieron la identificación del origen helmínhtico de todos los nódulos, en base a su forma ovalada y regular, la vasculatura periférica y la existencia de estructuras lineales internas hiperecogénicas 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 epidemiológic actual de la dirofilariosis humana.

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the nodules were asymptomatic. None have traveled outside the Rostov region in the previous past years.

By ultrasound examination, nodules were oval and regular-shaped with well-defined but jagged and fuzzy outer contours. The inner contents of the cyst appear hypoechoic. Inside the nodule there were clear internal echoes with linear winding hypechoic textures (Fig. 1A), sometimes with visible spontaneous writhing movement, detected in 14 out of 30 cases, indicating the presence of a live worm. Alive worms looked rich with hank-like multiplet continuing and sharp but fine tubular textures. Dead worm showed the same structures but they looked motionless, non-continuous, mostly blurred and broken, with areas of high and low echo density. Color Doppler examination detected that none of the nodules showed any signs of internal or polar vascularity, the blood vessels being present at the periphery of the parasitic cyst wall (Fig. 1B). Power Doppler examination confirmed the existence of blood flow only at the periphery of the nodules in the fibrotic capsule surrounding the worms (Fig. 1C) and adjacent tissues. The two nodules detected at retroocular location showed the same characteristic findings to those described in the rest of the nodules in both ultrasonograms and color and power Doppler examinations. These characteristics allowed the attribution of the origin of all nodules to a helmintic origin, probably *Dirofilaria*.

Surgical removal of the parasites revealed the presence of round worms with filiform appearance with lengths from 42 to 124 mm and diameters from 102 to 527 μm. They were sexually immature female worms in 28 out of 30 cases (Fig. 2A) and 2 immature male worms in the other 2 cases. PCR identified all of them as *D. repens* (Fig. 2B). Laboratory examination did not demonstrate any kind of abnormalities in conventional blood, urine and biochemistry tests.

**Discussion**

In spite of human dirofilarialiasis has been considered traditionally a rare disease, both the increase in clinical cases, mainly caused by *D. repens*, and the high seroprevalences revealed by serological surveys in humans suggest a high risk of infection by *Dirofilaria* species for people living in areas where dirofilarialiasis is endemic in dogs. At a global level the area with the highest increase in human dirofilarialialiasis cases is Europe where approximately 280 cases were reported until 2000, most of them in the Mediterranean countries. However, in the next 13 years the number of reported cases surpassed 2400 from which 2087 were reported in Ukraine and the Russian Federation. Moreover, with increasing frequency clinical cases are reported in Central and Northern European countries, previously considered non-endemic. These changes have been associated, among other factors, to the strong thermal anomalies occurring in the area in the last years and the wide stray dogs population not receiving chemoprophylaxis against *Dirofilaria*. The cases described here were reported in a single Hospital of Rostov-on-Don (Southwestern Russia), where previous epidemiological surveys revealed canine prevalences higher than 30%. This indicates a clear concordance among the existence of *Dirofilaria* in dogs and the detection of human cases. Nevertheless, there are high canine prevalences in other Southwestern Russian oblasts where only sporadic or no human cases have been reported, suggesting that the awareness of the medical community is also necessary for the correct diagnosis of human dirofilarialiasis.

Our series present similar epidemiological and clinical characteristics to those described previously by Pamplignon and Rivas in their review of the world literature, with some different aspects. Ocicular location is by far the most frequent, a fact that is in agreement with the high incidence of ocular cases reported in Russia as reviewed by Avdiukhina et al. Two of these cases appeared in retroocular location, having been one of them separately published to communicate this new anatomical location of *D. repens*. Because of the repetition of this presentation, it is necessary to warn the physicians about the possibility to find nodules in this anatomical location in the future. The frequency of genital location in our series (13.3%) is twice that found by Pamplignon and Rivas and appeared mostly in adolescents. There exist also differences in the distribution by gender because we have observed

**Table 1**

Main clinical and epidemiological characteristics of 30 cases of dirofilariosis (*Dirofilaria repens*) reported in Rostov-on-Don (Southwestern Russia), 2003–2013.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date</th>
<th>Patients’ sex (m/f)</th>
<th>Patients’ age (years)</th>
<th>Anatomical location</th>
<th>Clinical manifestation</th>
<th>Nodule size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.01.2003</td>
<td>f</td>
<td>34</td>
<td>Forehead</td>
<td>Subcutaneous nodule, painless</td>
<td>12 × 6</td>
</tr>
<tr>
<td>2</td>
<td>07.03.2003</td>
<td>f</td>
<td>37</td>
<td>Hip</td>
<td>Subcutaneous nodule, painless</td>
<td>14 × 8</td>
</tr>
<tr>
<td>3</td>
<td>23.04.2003</td>
<td>m</td>
<td>12</td>
<td>Testicular</td>
<td>Testicular mass, painless</td>
<td>17 × 8</td>
</tr>
<tr>
<td>4</td>
<td>28.05.2003</td>
<td>f</td>
<td>33</td>
<td>Forearm</td>
<td>Subcutaneous nodule, painless</td>
<td>15 × 8</td>
</tr>
<tr>
<td>5</td>
<td>09.01.2004</td>
<td>f</td>
<td>59</td>
<td>Ocular</td>
<td>Eyelid swelling, painful eye turns</td>
<td>11 × 6</td>
</tr>
<tr>
<td>6</td>
<td>17.02.2004</td>
<td>f</td>
<td>38</td>
<td>Groin</td>
<td>Subcutaneous nodule, painless</td>
<td>14 × 8</td>
</tr>
<tr>
<td>7</td>
<td>06.03.2004</td>
<td>m</td>
<td>31</td>
<td>Shoulder</td>
<td>Subcutaneous nodule, painless</td>
<td>15 × 7</td>
</tr>
<tr>
<td>8</td>
<td>27.10.2004</td>
<td>f</td>
<td>24</td>
<td>Retroocular</td>
<td>Periorbital edema</td>
<td>16 × 8</td>
</tr>
<tr>
<td>9</td>
<td>17.01.2005</td>
<td>f</td>
<td>39</td>
<td>Hip</td>
<td>Subcutaneous nodule, itch</td>
<td>14 × 6</td>
</tr>
<tr>
<td>10</td>
<td>22.04.2005</td>
<td>f</td>
<td>53</td>
<td>Ocular</td>
<td>Eyelid swelling, tearing</td>
<td>10 × 5</td>
</tr>
<tr>
<td>11</td>
<td>28.10.2005</td>
<td>m</td>
<td>14</td>
<td>Testicular</td>
<td>Testicular mass, painless</td>
<td>15 × 8</td>
</tr>
<tr>
<td>12</td>
<td>15.02.2006</td>
<td>f</td>
<td>32</td>
<td>Cheek</td>
<td>Subcutaneous nodule</td>
<td>13 × 7</td>
</tr>
<tr>
<td>13</td>
<td>07.12.2006</td>
<td>f</td>
<td>37</td>
<td>Ocular</td>
<td>Eyelid swelling, burning sensation</td>
<td>10 × 5</td>
</tr>
<tr>
<td>14</td>
<td>26.01.2007</td>
<td>m</td>
<td>45</td>
<td>Ocular</td>
<td>Eyelid swelling, itch</td>
<td>11 × 6</td>
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<tr>
<td>15</td>
<td>14.04.2007</td>
<td>f</td>
<td>38</td>
<td>Zygomatic</td>
<td>Subcutaneous nodule, painless</td>
<td>12 × 6</td>
</tr>
<tr>
<td>16</td>
<td>03.05.2007</td>
<td>f</td>
<td>31</td>
<td>Hip</td>
<td>Subcutaneous nodule, painless</td>
<td>13 × 7</td>
</tr>
<tr>
<td>17</td>
<td>07.10.2007</td>
<td>m</td>
<td>7</td>
<td>Testicular</td>
<td>Testicular mass, painless</td>
<td>16 × 7</td>
</tr>
<tr>
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<td>16.03.2008</td>
<td>m</td>
<td>34</td>
<td>Forearm</td>
<td>Subcutaneous nodule, painless</td>
<td>14 × 8</td>
</tr>
<tr>
<td>19</td>
<td>11.11.2008</td>
<td>f</td>
<td>24</td>
<td>Ocular</td>
<td>Eyelid swelling, conjunctivitis</td>
<td>11 × 6</td>
</tr>
<tr>
<td>20</td>
<td>08.02.2009</td>
<td>f</td>
<td>31</td>
<td>Ocular</td>
<td>Eyelid swelling, burning</td>
<td>10 × 5</td>
</tr>
<tr>
<td>21</td>
<td>21.10.2009</td>
<td>f</td>
<td>56</td>
<td>Temporal</td>
<td>Subcutaneous nodule, painless</td>
<td>14 × 7</td>
</tr>
<tr>
<td>22</td>
<td>09.03.2010</td>
<td>f</td>
<td>30</td>
<td>Periorbital</td>
<td>Subcutaneous nodule, painless</td>
<td>11 × 6</td>
</tr>
<tr>
<td>23</td>
<td>17.10.2010</td>
<td>f</td>
<td>58</td>
<td>Groin</td>
<td>Subcutaneous nodule, painless</td>
<td>12 × 7</td>
</tr>
<tr>
<td>24</td>
<td>18.04.2011</td>
<td>f</td>
<td>29</td>
<td>Forearm</td>
<td>Subcutaneous nodule, painless</td>
<td>14 × 6</td>
</tr>
<tr>
<td>25</td>
<td>15.09.2011</td>
<td>f</td>
<td>21</td>
<td>Retroocular</td>
<td>Intermittent periorbital edema</td>
<td>16 × 8</td>
</tr>
<tr>
<td>26</td>
<td>14.12.2011</td>
<td>m</td>
<td>35</td>
<td>Abdominal wall</td>
<td>Subcutaneous nodule, painless</td>
<td>18 × 8</td>
</tr>
<tr>
<td>27</td>
<td>30.01.2012</td>
<td>m</td>
<td>37</td>
<td>Testicular</td>
<td>Left testicular edema, skin redness</td>
<td>17 × 8</td>
</tr>
<tr>
<td>28</td>
<td>03.03.2012</td>
<td>f</td>
<td>54</td>
<td>Ocular</td>
<td>Eyelid redness, itch</td>
<td>10 × 5</td>
</tr>
<tr>
<td>29</td>
<td>04.07.2012</td>
<td>m</td>
<td>22</td>
<td>Abdominal wall</td>
<td>Subcutaneous nodule, painless</td>
<td>14 × 7</td>
</tr>
<tr>
<td>30</td>
<td>13.06.2013</td>
<td>f</td>
<td>45</td>
<td>Ocular</td>
<td>Eyelid redness, itch</td>
<td>12 × 6</td>
</tr>
</tbody>
</table>
70.0% of incidence in women, clearly higher than 55.4% reviewed by Pampiglione and Rivasi.17

Detection of Dirofilaria nodules frequently causes the initial suspicion of a malignant origin, in spite of their benign character.3 Thus differential diagnosis is an essential step of the management of human dirofilariosis.7,19 Diagnosis of subcutaneous dirofilariosis is post-operative in most cases, causing moderate to serious injury to patients, mainly when nodules appear in internal, intraocular or retroocular locations.8,9 To avoid unnecessary damages and delay during the diagnostic procedure, efficient non-invasive techniques can be applied. The results obtained in the wide series of cases presented here consistently demonstrate the ability of ultrasounds combined to color and power Doppler technique in the presumptive attribution of these nodules to a dirofilariotic origin, in both superficial and deep locations. While in canine and feline cardiopulmonary dirofilariosis echocardiographic techniques are routinely used for the diagnosis and evaluation of the clinical situation of the hosts,20 these techniques have been successfully applied in the definitive diagnosis of human dirofilariosis only in very few cases21–23, or were used only to guide fine needle

**Fig. 1.** Representative images from a B-scan ultrasound (A, case no. 27) and color and power Doppler sonography (B and C) examination of a parasitic nodules (both from case no. 22). Parasite appears as multiple parallel hyperechoic linear structures (rail track pattern) inside the cysts and blood flow is located in the periphery of the capsule.

**Fig. 2.** Subcutaneous nodule containing a female worm surgically removed from a patient. The scale bar is in cm (A). Representative image of the PCR analysis of the DNA extracted from different worms after the surgical removal of the nodules (B). The dirofilariotic nodules were located in subcutaneous tissue of the shoulder (case no. 7) (Lane 1), ocular area (case no. 10) (Lane 2), testicular (case no. 17) (Lane 3), abdominal wall (case no. 26) (Lane 4) and. M, 100 bp DNA marker; C+, positive control; C−, negative control.
aspiration biopsy.\textsuperscript{24,25} The identification of the helminthic origin of the nodules, discarding a malignant tumor, was achieved according to morphological aspect of the nodules, the presence of worms which appear as parallel hyperechotic lines inside them and even their movements in nearly half of the cases and the peripheral location of blood vessels in Dirofilaria nodules, unlike what happens in malignant tumors, where the vascularization appears inside the nodule. Specific adscription to D. repens and thus confirmation of suspicions acquired by ultrasounds and Doppler examinations was performed in all cases by PCR once removed the nodules or the worm themselves. Nevertheless, once identified the dirofilariotic origin of the nodules and discarded a malignant origin, this is a secondary matter\textsuperscript{19} that must be performed when diagnosis make necessary the removal of the nodules or worms.

In conclusion, we present here the report of a wide series of cases of human dirofilariosis in a hospital of a hyperendemic area of dirofilariosis in Southwestern Russia by means of image and molecular techniques. These techniques allowed the identification of dirofilariotic origin of all nodules located in both superficial and deeper locations. Results of epidemiological surveys and estimations of prediction models\textsuperscript{6,20} suggest a spreading of dirofilariosis both in animal reservoir and human populations of Northern and Eastern European countries. Thus human subcutaneous/ocular dirofilariosis would be diagnosed with increasing frequency in the nearest future in Europe.

**Conflict of interest**

The authors have no conflict of interest to declare.

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