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## Final Program

### 2 July, 2014 – Wednesday

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<tr>
<td>17:00-20:00</td>
<td>Registration</td>
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<tr>
<td>18:00-20:00</td>
<td>Get Together (120 min)</td>
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### 3 July, 2014 – Thursday

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<tr>
<td>08:00-09:00</td>
<td>Registration</td>
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<tr>
<td>09:00-09:30</td>
<td>Opening Ceremony and Welcome Addresses (30 min)</td>
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<tr>
<td>09:30-09:50</td>
<td>Plenary lectures - Invited Speakers</td>
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<tr>
<td></td>
<td><strong>TOPIC: 1. DIROFILARIOSES</strong></td>
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<tr>
<td></td>
<td>Chairpersons: Róbert Farkas, Fernando Simón</td>
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<tr>
<td>09:30-09:50</td>
<td>NEWS ABOUT HUMAN DIROFILARIOsis</td>
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<tr>
<td></td>
<td>Fernando Simón, Vladimir Kartashev, Mar Siles-Lucas, Rodrigo Morchón,</td>
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<td></td>
<td>Javier González-Miguel</td>
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<tr>
<td></td>
<td>(20 min)</td>
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<tr>
<td>09:50-10:20</td>
<td>FOCUS ON ESCCAP GUIDELINES FOR DIAGNOSIS, PREVENTION AND CONTROL OF</td>
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<tr>
<td></td>
<td>DIROFILARIA INFECTION IN DOGS AND CATS</td>
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<tr>
<td></td>
<td>Claudio Genchi</td>
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<tr>
<td></td>
<td>(30 min)</td>
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<tr>
<td>10:20-10:35</td>
<td>KNOW YOUR (DIROFILARIA) ENEMY … AT THE ULTRASTRUCTURAL LEVEL</td>
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<tr>
<td></td>
<td>Wieslaw J. Kozek</td>
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<td></td>
<td>(15 min)</td>
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<tr>
<td>09:30-10:30</td>
<td>Coffee break and</td>
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<tr>
<td></td>
<td>Poster session-Moderator: Gábor Földvari</td>
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<tr>
<td></td>
<td>(55 min)</td>
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<tr>
<td>11:30-12:00</td>
<td>Plenary lectures - Invited Speakers</td>
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<td></td>
<td><strong>TOPIC: 1. DIROFILARIOSES</strong></td>
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<tr>
<td></td>
<td>Chairpersons: Claudio Genchi, Georg Gerhard Duscher</td>
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<tr>
<td>11:30-12:10</td>
<td>RADIOLOGY VS ECHOCARDIOGRAPHY. HOW TO SQUEEZE THE BEST FROM EACH OF</td>
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<td>THEM FOR STAGING, MONITORING AND CHOOSING THE BEST THERAPY IN</td>
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<td>HEARTWORM DISEASE IN DOGS</td>
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<tr>
<td></td>
<td>Luigi Venco</td>
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<td></td>
<td>(40 min)</td>
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<td>12:10-12:40</td>
<td>IMPORTANCE OF MICROFILARIAL CONTROL AND OPTIONS FOR REMOVAL WITH</td>
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<tr>
<td></td>
<td>10% IMIDACLOPRID + 2.5% MOXIDECTIN</td>
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<td></td>
<td>Dwight D. Bowman</td>
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<td>(30 min)</td>
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<tr>
<td>12:40-13:10</td>
<td><strong>HUMAN DIROFILARIOsis - MORbidity Trends in the Former USSR</strong></td>
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<tr>
<td></td>
<td>Vladimir Kartashev, Tatiana Tverdokhlebova, Eduard Yagovkin, Yuri Ambalov,</td>
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<td>Tamara Pavlikovskaya, Olga Sagach, Svetlana Nikolaenko, Nina Chizh,</td>
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<td>Zh Chandler Bekshin, Zhanna Shapiyeva, Alla Korzan, Andrey Vedenkov,</td>
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<td></td>
<td>Alexander Afonin, Luis Simon, Fernando Simón</td>
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<tr>
<td></td>
<td><em>(30 min)</em></td>
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<tr>
<td>13:10-13:40</td>
<td><strong>Canine and Human Dirofilaria Infections: What is New in the Balkan Peninsula</strong></td>
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<tr>
<td></td>
<td>Suzana Otašević, Aleksandar Tasić, Simona Gabrielli, Marija Trenkić Božinović,</td>
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<td></td>
<td>Gabriella Cancrini</td>
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<td><em>(30 min)</em></td>
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<td>13:40-14:40</td>
<td>Lunch</td>
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<td><em>(60 min)</em></td>
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<tr>
<td>14:40-14:55</td>
<td><strong>Epidemiological View About Dirofilaria Infections in Dogs of Hungary</strong></td>
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<tr>
<td></td>
<td>Olga Jacsó, Gabriella Kiss, Diána Krassóvári, Hubert János Kiss,</td>
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<td>Mónika Gyurkovszky, Éva Fok</td>
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<td><em>(15 min)</em></td>
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<td>14:55-15:10</td>
<td><strong>Dirofilaria Repens and D. Immitis in Hungary. Experience of a Commercial Laboratory</strong></td>
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<td>Nándor Balogh, Viktória Kunos, Gyula László Rabnecz</td>
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<td><em>(15 min)</em></td>
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<td>15:10-15:25</td>
<td><strong>Human Dirofilaria Repens Infection Diagnosed in National Center for Epidemiology, Hungary</strong></td>
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<td>István Kucsera, József Danka, Erika Orosz</td>
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<td><em>(15 min)</em></td>
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<td>15:25-15:40</td>
<td><strong>Canine Dirofilariosis in Slovakia – The Results of 8-Year Epidemiological Research</strong></td>
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<td>Martina Miterpáková, Adriana Iglódyová, Daniela Antolová, Zuzana Hurníková</td>
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<td>15:40-15:55</td>
<td><strong>Filaroid Infections in Dogs from Romania: A Broader View</strong></td>
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<td></td>
<td>Angela Monica Ionică, Ioana Adriana Matei, Viorica Mircean,</td>
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<td>Mirabela Oana Dumitrache, Giada Annoscia, Domenico Otranto,</td>
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<td>David Modry, Andrei Daniel Mihalca</td>
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<td><em>(15 min)</em></td>
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<tr>
<td>15:55-16:10</td>
<td><strong>Epidemiologically Study About Natural Infestation with Dirofilaria in Shelters Located in the Southern Part of Romania</strong></td>
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<td>Cristian-Ionut Florea, Stefan Olaru, Anca Dobrica, Poliana Tudor</td>
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<tr>
<td>16:10-16:25</td>
<td><strong>Epidemiology of Dirofilariosis in Dogs in Greece: Previous and Latest Information</strong>&lt;br&gt; Anastasia Diakou, Emmanouil Kapantaidakis (15 min)</td>
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<td>16:25-17:00</td>
<td><strong>ZOETIS Coffee break and Poster session</strong>&lt;br&gt; <strong>Moderator:</strong> László Békési (35 min)</td>
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<tr>
<td>17:00-17:15</td>
<td><strong>Invited speaker and Free communications</strong>&lt;br&gt; <strong>TOPIC: 1. DIROFILARIOSES</strong>&lt;br&gt; <strong>Chairpersons:</strong> Herbert Auer, István Kucsera</td>
</tr>
<tr>
<td>17:00-17:15</td>
<td><strong>Human Dirofilariosis - Clinical Presentation and Diagnosis</strong>&lt;br&gt; Vladimir Kartashev, Tatiana Tverdokhlebova, Eduard Yagovkin, Nikolay Bastrikov, Denis Donssov, Yuri Ambalov, Tamara Pavlikovskaya, Olga Sagach, Svetlana Nikolaenko, Nina Chizh, Zhodarbebek Bekshin, Zhanna Shapiyeva, Alla Korzan, Andrey Vedenkov, Boris Ilyasov, Javier González-Miguel, Rodrigo Mochón, Fernando Simón (15 min)</td>
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<tr>
<td>17:15-17:30</td>
<td><strong>Overview of Dirofilariosis in Serbia during the last ten years 2004-2014 and current status of the disease</strong>&lt;br&gt; Sara Savić, Branka Vidic, Pajkovic Dusan, Ljubica Spasojevic-Kosic, Strahinja Medic, Aleksandar Potkonjak, Suzana Otašević (15 min)</td>
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<tr>
<td>17:30-17:45</td>
<td><strong>Updating the Prevalence of Canine Dirofilariosis in Pet Dogs in Novi Sad, Voivodina, Serbia</strong>&lt;br&gt; Ljubica Spasojevic Kosić, Stanislav Simin, Vesna Lalosević, Ljiljana Kuruca, Sandra Nikolić, Danijela Nerac (15 min)</td>
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<tr>
<td>17:45-18:00</td>
<td><strong>Canine Filarioïdosis in Lisbon, Portugal, over a 14-Year Period: Prevalence, Diagnosis and Routine Laboratory Approaches</strong>&lt;br&gt; Ana Margarida Alho, Lidia Gomes, Isabel Pereira da Fonseca, José Meireles, Luis Madeira de Carvalho (15 min)</td>
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<tr>
<td>18:00-18:15</td>
<td><strong>Dirofilaria Repens and Dirofilaria Immitis in Austria</strong>&lt;br&gt; Hans-Peter Fuehrer, Herbert Auer, Katja Silbermayr, Georg Gerhard Duscher (15 min)</td>
</tr>
<tr>
<td>18:30 – late night</td>
<td><strong>Horse show and dinner at Lázár Equestrian Park (Domonyvölgy)</strong>&lt;br&gt; Transportation by bus is provided (the journey takes approx. 1 hour).&lt;br&gt; Meeting time and point: at 18:30 at Szent István University, Faculty of Veterinary Science (1078 Budapest, István u. 2.)&lt;br&gt; Between 22:00-23:00 buses continuously leave for Budapest taking guests back to the official FEDAD Hotels.</td>
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<td>Time</td>
<td>Session</td>
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<tr>
<td>08:30-09:10</td>
<td><strong>WOLBACHIA IN CANINE AND FELINE HEARTWORM INFECTION</strong></td>
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<tr>
<td>09:10-09:50</td>
<td><strong>FELINE DIROFILARIOSIS: PATHOGENESIS, SIGNS, TREATMENT AND PROGNOSIS</strong></td>
</tr>
<tr>
<td>09:50-10:35</td>
<td><strong>RESISTANCE AND HEARTWORM PREVENTIVES: HISTORICAL PERSPECTIVE AND OUTLOOK FOR THE US AND OTHER REGIONS</strong></td>
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<tr>
<td>10:35-11:15</td>
<td>Coffee break and Poster session</td>
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<tr>
<td>11:15-11:30</td>
<td>**PREVENTION OF AN EXPERIMENTAL <strong>DIROFILARIA REPENS INFECTION WITH A SPOT ON COMBINATION OF MOXIDECTIN 2,5% /IMIDACLOPRID 10% (ADVOCATE®, ADVANTAGE® MULTI)</strong></td>
</tr>
<tr>
<td>11:30-11:45</td>
<td><strong>NODULAR PRESENTATION OF</strong>DIROFILARIA REPENS IN A CAT MIMICKING SOFT TISSUE SARCOMA**</td>
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<tr>
<td>11:45-12:00</td>
<td><strong>ANALYSIS OF LABORATORY PARAMETERS IN CASE OF DIROFILARIA REPENS INFECTION</strong></td>
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<td>12:00-12:15</td>
<td><strong>SEROPREVALENCE OF FELINE HEARTWORM (DIROFILARIA IMMITIS) DISEASE IN CATS OF CENTRAL PORTUGAL</strong></td>
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<td>12:15-12:30</td>
<td><strong>AN ALTERNATIVE METHOD FOR MECHANICAL REMOVAL OF DIROFILARIA IMMITIS IN DOGS USING A HOMEMADE SNARE</strong></td>
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<td>Session</td>
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<tr>
<td>12:30-12:45</td>
<td>MICROFILARAEMIA AND ANTIGENEMIA IN DOGS WITH NATURAL HEARTWORM INFECTION TREATED WITH COMBINATION OF DOXYCYCLIN AND IVERMECTIN - PRELIMINARY RESULTS</td>
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<td>12:45-13:00</td>
<td>MOLECULAR DETECTION OF CERCOPHITIFILARIA SPP. IN IXODID TICKS</td>
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<td>13:00-14:00</td>
<td>Lunch</td>
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<tr>
<td>14:00-14:05</td>
<td>Welcome and Opening remarks</td>
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<td>14:05-14:30</td>
<td>SHORT COMMUNICATIONS – ORAL PRESENTATIONS I.</td>
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<tr>
<td>14:05-14:30</td>
<td>ANGIOSTRONGYLUS VASORUM - NOVELTIES ABOUT EPIDEMIOLOGY &amp; DIAGNOSTIC TOOLS</td>
</tr>
<tr>
<td>14:30-14:45</td>
<td>ANGIO DETECT™ - A NEW TOOL FOR THE RAPID DETECTION OF ANGIOSTRONGYLUS VASORUM INFECTION</td>
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<tr>
<td>14:45-15:00</td>
<td>ANGIOSTRONGYLUS ‘HOTSPOTS’ IN THE UK – PRELIMINARY EPIDEMIOLOGICAL INVESTIGATIONS</td>
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<td>15:00-15:15</td>
<td>OCCURRENCE OF ANGIOSTRONGYLUS VASORUM INFECTIONS IN DOGS WITH A RELEVANT CLINICAL PICTURE IN DENMARK</td>
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<td>15:15-15:30</td>
<td>LUNGWORM (ANGIOSTRONGYLUS VASORUM, CRENOSOMA VULPIS, EUCOLEUS AEROPHILA) INFECTIONS IN RED FOX POPULATIONS IN SOUTH WEST GERMANY</td>
</tr>
<tr>
<td>15:30-16:00</td>
<td>Coffee break and Poster presentations within the framework of the ‘3rd Bayer Angiostrongylosis Forum’</td>
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<tr>
<td>16:00-16:15</td>
<td><strong>UK: NORTHWARD SPREAD OF <em>ANGIOSTRONGYLUS VASORUM</em> – DATA FROM DOGS AND FOXES</strong></td>
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<tr>
<td>16:15-16:30</td>
<td><strong>DIAGNOSIS OF FRENCH HEARTWORM AND LUNGWORMS IN SWEDISH DOGS: RESULTS FROM 2011-2013</strong></td>
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<td>16:30-16:45</td>
<td><strong>RESPIRATORY NEMATODES IN DOGS FROM CENTRAL ITALY</strong></td>
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<tr>
<td>16:45-17:00</td>
<td><strong>AEUROSTRONGYLUS ABSTRUSUS: NOT JUST PNEUMONIA; TWO CLINICAL SYNDROMES ASSOCIATED WITH A. ABSTRUSUS</strong></td>
</tr>
<tr>
<td>17:00-17:15</td>
<td><strong>ANGIOSTRONGYLUS VASORUM TRESPASSED THE BORDERS OF SLOVAKIA</strong></td>
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<td>17:15-18:15</td>
<td><strong>General Assembly</strong></td>
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<td><strong>Closing remarks</strong></td>
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### POSTERS

**Topic: 1. DIROFILARIOSES**

#### 1.1. NEW DATA ABOUT THE EPIDEMIOLOGY IN ANIMALS IN EUROPE

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<th>Authors</th>
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<td>P1</td>
<td>CANINE FILARIOID INFECTIONS IN AN AREA OF NORTH-WEST ITALY (LIGURIA) TRADITIONALLY CONSIDERED FREE FROM THE DISEASE</td>
<td>Marta Magi, Lisa Guardone, Maria Cristina Prati, Walter Mignone, Giorgia Tozzini &amp; Fabio Macchioni</td>
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<td>P2</td>
<td>CASES OF DIROFILARIOsis IN DOGS AND CATS IN BUCHAREST AND SURROUNDING AREAS</td>
<td>Raluca Ioana Rizac, Iulia-Alexandra Paraschiv, Andrei Constantin Stoian, Emilia Ciobotaru, Teodoru Soare, Dumitru Militaru &amp; Manuela Militaru</td>
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<tr>
<td>P3</td>
<td>PREVALENCE OF DIROFILARIA IMMITIS IN STRAY DOGS IN ERZURUM PROVINCE, TURKEY</td>
<td>Hamza Avcioglu &amp; Esin Guven</td>
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<td>P4</td>
<td>PREVALENCE OF DIROFILARIA IMMITIS IN PET AND STRAY DOGS IN BELGRADE AREA IN PERIOD 2012-2013</td>
<td>Ivan Pavlović, Vladimir Terzin, Bratislav Stanković, Dragan Petković, Ljubomir Curčin, Vladimir Antić, Dragana Terzin &amp; Klara Curčin</td>
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<tr>
<td>P5</td>
<td>SEROLOGICAL SURVEY ON CANINE HEARTWORM (DIROFILARIA IMMITIS) INFECTION AND OTHER VECTOR-BORNE PATHOGENS IN DOGS FROM BUCHAREST, ROMANIA</td>
<td>Gina Teodora Girdan, Mariana Ioniță &amp; Ioan Liviu Mitrea</td>
</tr>
<tr>
<td>P6</td>
<td>MAPPING AND MODELING DIROFILARIA INFECTIONS IN EUROPE</td>
<td>Vincenzo Musella, Laura Rinaldi, Giuseppe Marzatico, Michele Mortarino, Giuseppe Cringoli &amp; Claudio Genchi</td>
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#### 1.2. ZOONOTIC ASPECTS OF DIROFILARIA REPENS AND D. IMMITIS

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<th>Authors</th>
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<td>P7</td>
<td>HUMAN DIROFILARIA REPENS INFECTION IN UKRAINE (1997–2013)</td>
<td>Ruslan Salamatin, Tamara Pavlikovska, Olga Sagach, Svitlana Nikolayenko, Vadim Kornyushin, Vitaliy Kharchenko, Aleksander Masny, Danuta Cielecka, Joanna Koniczna-Salamatin, David Bruce Conn &amp; Elzbieta Golab</td>
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<td>P8</td>
<td>FURTHER CASES OF HUMAN DIROFILARIOsis DIAGNOSED IN SOUTH-EAST SERBIA</td>
<td>Simona Gabrielli, Aleksandar Tasić, Marija Trenkić-Božinović, Marina Ignjatović, Suzana Otašević &amp; Gabriella Cancrini</td>
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<tr>
<td>P9</td>
<td>HUMAN DIROFILARIASIS: FOUR NEW SERBIAN CASES CAUSED BY DIROFILARIA REPENS</td>
<td>Aleksandar M Džamić, Jelica Pantelić, Igor Kovačević, Jelena Karadžić, Zorica Dakić &amp; Ivana Čolović Čalovski</td>
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<tr>
<td>P10</td>
<td>NEWER CASE OF HUMAN SUBCUTANEOUS DIROFILARIOsis IN HUNGARY</td>
<td>Ilona Dóczi, László Bereczki, Balázs Bende &amp; Erika Kis</td>
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<tr>
<td>1.3. DIAGNOSTIC METHODS TO DIFFERENTIATE CUTANEOUS AND IMMITIS DIROFILARIOSIS</td>
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| **P12** | DIRECT PCR FOR DETECTION OF DIFFERENT 
*DIROFILARIA* SPP. AND STAGES IN DIFFERENT SUBSTRATES |
| Katja Silbermayr, Barbara Eigner, Georg Gerhard Duscher, Anja Joachim & Hans-Peter Fuehrer |
| **P13** | THE USE OF SCREENING BLOOD-FED MOSQUITOES FOR THE 
DIAGNOSIS OF *DIROFILARIA* |
| Carina Zittra, Zsannett Kocziha, Szilárd Pinnyei, Katrin Kieser, Alice Laciny, Barbara Eigner, Katja Silbermayr, Georg Duscher, Éva Fok & Hans-Peter Fuehrer |
| **P14** | MICROANATOMICAL COMPARISON OF HUMAN AND ANIMAL 
*DIROFILARIA REPENS* ISOLATED IN IRAN |
| Seyed Mahmoud Sadjjadi & Nafiseh Ghobakhlou |
| **P15** | MOLECULAR DIAGNOSTICS OF SOME MICROFILARIA SPECIES IN 
UPPER EGYPT REGION |
| Nasr M. El Bahy |

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| **P16** | MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE 
PLASMINOGEN-BINDING PROTEINS OF *DIROFILARIA IMMITIS* ACT, 
FBAL, GAPDH AND GAL |
| Javier González-Miguel, Laura Martin, Alice Rossi, Rodrigo Morchón, Isabel Mellado, Mar Siles-Lucas, Ana Oleaga & Fernando Simón |
| **P17** | CAN THE ACTIVATION OF PLASMINOGEN/PLASMIN SYSTEM OF 
THE HOST BY METABOLIC PRODUCTS OF *DIROFILARIA IMMITIS* 
PARTICIPATE IN HEARTWORM DISEASE ENDARTERITIS? |
| Javier González-Miguel, Carmen Larrazabal, Rodrigo Morchón, Elena Carreton, Jose Alberto Montoya-Alonso & Fernando Simón |

**Topic: 2. OTHER FILARIOIDS IN CARNIVORES**

| **P18** | *CERCOPITHEFILARIA BAINAE* IN A DOG SUFFERING FOR CHRONIC 
POLYARTHRTISIS |
| Simona Gabrielli, Alessio Giannelli, Emanuele Brianti, Filipe Dantas-Torres, Massimiliano Bufalini, Maurizio Fraulo, Francesco La Torre, Rafale A. N. Ramos, Maria Stefania Latrofa, Gabriella Cancrini & Domenico Otranto |
| **P19** | STUDIES ON *DIROFILARIA REPENS* AND *ACANTHOCEHILONEMA RECONDITUM* IN STRAY DOGS IN ERZURUM PROVINCE, TURKEY |
| Esin Guven & Hamza Avcioglu |
Topic: 3. ANGIOSTRONGYLOSIS

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Invited speakers

Dr. Clarke Atkins, DVM

ACADEMIC AND ADMINISTRATIVE POSITIONS
He is the Jane Lewis Seaks Distinguished Professor of Companion Animal Medicine (Emeritus) at North Carolina State University and 2004 Norden Outstanding Teacher Award Winner, is board-certified by the ACVIM (Medicine & Cardiology).

RESEARCH ACTIVITIES AND SCIENTIFIC PAPERS WRITING
His research involves canine and feline heartworm disease and treatment of cardiovascular disease in dogs, cats, and horses. He has over 150 publications and has provided well over 1000 hours of continuing education in the U.S. and many countries around the world.

Dr. Atkins continues to be active speaking and carrying out clinically-oriented research in retirement.

Dr. Byron L. Blagburn, MS, PHD

ACADEMIC AND ADMINISTRATIVE POSITIONS
Byron L. Blagburn holds the appointment of Distinguished University Professor at the Auburn University College of Veterinary Medicine. He received his doctorate in parasitology from the University of Illinois College of Veterinary Medicine, and is an Honorary Diplomate of the American College of Microbiology (Parasitology).

TEACHING ACTIVITY
He instructs first, third, and fourth-year students of veterinary medicine, directs graduate student research, serves as director of the clinical parasitology diagnostic laboratory and oversees a research program which focuses on parasite-induced diseases of companion animals.

SCHOLARSHIPS AND AWARDS
He is Past President of the American Association of Veterinary Parasitologists, the Southern Conference on Animal Parasites, the Southeastern Society of Parasitologists, and the Companion Animal Parasite Council (CAPC).

He has served as an Associate Editor for the Journal of Parasitology and has served on the editorial boards of Veterinary Parasitology, Veterinary Therapeutics, The International Journal of Applied Research in Veterinary Medicine, and The Journal of Eukaryotic Microbiology. Dr. Blagburn is a recipient of the American Association of Veterinary Parasitologist’s Distinguished Veterinary Parasitologist Award, the Pfizer Award for Research Excellence (1987 and 2010), and the Auburn University Student Government Association “Teacher of the Year” Award.
Dr. Dwight D. Bowman, MS, PhD, DACVM (Honorary)

ACADEMIC AND ADMINISTRATIVE POSITIONS
Dr. Bowman graduated with honors in Biology from Hiram, College in Hiram, OH, in 1974. He received his MS and PhD degrees in parasitology from Tulane University in New Orleans, in 1976 and 1983. He was a postdoctoral scientist/lecturer at the School of Veterinary Medicine of the University of Wisconsin Madison from 1984-1987. He joined the faculty of the College of Veterinary Medicine at Cornell University, Ithaca, NY, in 1987, and is currently a Full Professor in Parasitology

TEACHING ACTIVITY
He teaches courses in the veterinary, graduate, and undergraduate curricula of the Cornell University, Ithaca, NY.

SCHOLARSHIPS AND AWARDS
In 2008, he was awarded the Distinguished Veterinary Parasitologist Award by the American Association of Veterinary Parasitologists. In 2009, he was awarded the Chancellor's Award for Excellence in Teaching from the State University of New York. In 2009, he was also awarded Cornell University’s College of Veterinary Medicine’s first Community Service Award, and a recipient in 2010 of the Kaplan Family Distinguished Faculty Fellowship in Service-Learning. He is an honorary Diplomate in the Parasitology section of the American College of Veterinary Microbiologists. He is currently president of the American Association of Veterinary Parasitologists. He was a founding member of the Companion Animal Parasite Council which officially began in 2002, and has just completed a two-year term as president.

RESEARCH ACTIVITIES AND SCIENTIFIC PAPERS WRITING
As a parasitologist, the focus of his research has been on the biology of parasitic infections, testing of various agents for efficacy against parasites, disinfection of parasites in manures and sewage sludges, and improved diagnostics. He is author of 5 textbooks on parasitology including Georgis’ Parasitology for Veterinarians. He has published around 200 peer-reviewed papers on the topics of veterinary parasitology, and in addition to being an ad hoc reviewer for over 20 journals, serves or has served on the Editorial Boards of Journal of Zoo and Wildlife Medicine, Comparative Parasitology, Veterinary Parasitology, International Journal for Parasitology, and the Compendium of Continuing Education for the Practicing Veterinarian.
Dr. Claudio Genchi, Professor

PERSONAL DATA
Birth place: Luino, Italy
Full Professor of Parasitic Disease, University of Milan, Italy

ACADEMIC AND ADMINISTRATIVE POSITIONS
President of the Direction Committee of the Faculty

SCHOLARSHIP AND AWARDS
Founding Member and Past-President of the European Veterinary College of Parasitology (EVPC)
Honorary Membership American Heartworm Society (AHS) (2007–)
President of Italian Society of Parasitology (2000–2008)
Laurea Honoris Causa, Faculty of Veterinary Medicine, University of Agronomic Sciences, Bucharest (2010)

TEACHING ACTIVITY
Currently, he is teaching Parasitic Disease and Zoonotic Parasitic Infections in the curriculum of Veterinary Medicine and in several Specialization Courses of the Faculty of Veterinary Medicine and Medical Schools of the University of Milan. In 1989 and 1992 he was Invited Professor in Epidemiology of Heartworm Disease in Europe, College of Veterinary Medicine, Faculty of Parasitology, The University of Georgia, Athens, GE, USA. In 1988 he was Visiting Professor in charge of the Italian Ministry of Foreign Affairs, Xining University, Qinghai Animal Husbandry and Veterinary College, Xining City, Qinghai Province (China).

RESEARCH ACTIVITIES AND SCIENTIFIC PAPERS
Primary research areas are the molecular approach to the biology, genetics, immunology and pathogenesis of parasitic diseases, including vector-borne infections (filarial and tick-borne diseases), experimental chemotherapy and control of endo- and ectoparasites and zoonotic infections. He is leader of one of the research teams involved in the study of Wolbachia, the bacterial endosymbiont of filarial nematodes. His team first identified these bacteria by molecular studies in D. immitis in 1995.
Author and Co-author of more than 200 peer reviewed articles (indexed in PubMed) and of 6 scientific and student books.
Dr. Vladimir Vasilyevich Kartashev, Associate Professor, Department of Infectious Diseases, Rostov State Medical University, Rostov-na-Donu, Russia

PERSONAL DATA
1976 - 1982  Post-graduate scientific research and practical activity in clinical medicine, lecturer of infectious diseases.
1995 - IREX fellowship, IREX exchange program GWU School of Medicine and Health Science, George Washington University, Washington, D.C., USA.
2001, 2002, 2003 - Visiting Professor, St.-Johhans Hospital, Salzburg. Grants of the American-Austrian Foundation, Salzburg, Austria
2006 – Visiting Professor, Japanese National Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan (6 months).
2010, 2011 – Visiting Professor. University of Salamanca, Spain

ACADEMIC AND ADMINISTRATIVE POSITIONS
1974 - Academic Rank of Assistant Professor.
1982 - Scientific Degree of Doctor of Medicine (PhD).
1985 - Academic Rank of Associate Professor.
1993 - Supreme Qualification in Medicine, Russian Ministry of Health.
1993 - Licensed specialist in Infectious Diseases and Parasitology.
2011 - Scientific Degree of Full Doctor of Medicine, title of Professor of Medicine.

EXPERIENCE
1976-1985 - Assistant Professor of the Department of Infectious Diseases, Rostov State Medical Institute. Physician of the Department of Infectious Diseases. Central Municipal Hospital. Consultant at the Hospital of the Rostov Medical Institute.
1985-present - Associate Professor of the Department of Infectious Diseases Rostov State Medical University. Consultant of the Rostov Central Municipal Hospital and Rostov Central Regional Hospital. Consultant of the
Hospital of Rostov Medical University.

Research fields: Human and animal dirofilariosis. Waterborne parasitic diseases. Parasitic diseases in AIDS.


Publications: 125 published papers, 6 published monographs.

Wieslaw J. Kozek, Ph.D.

PERSONAL DATA
He was born in Poland. He obtained his B.S. degree from Canisius College, in Buffalo, N.Y., and both M.S. and Ph.D. degrees from Tulane University in New Orleans, LA.

ACADEMIC AND ADMINISTRATIVE POSITIONS
After completing postdoctoral fellowships at the University of Chicago and the University of Florida (Gainesville), he was an Investigator at the University of California Primate Research Center (Davis) and subsequently directed studies on human filariasis - a component project of Tulane University ICIDR Program - in Colombia, South America. Since 1984, he has been a member of the Department of Microbiology and Medical Zoology at the Medical Sciences Campus of the University of Puerto Rico in San Juan, where he currently holds the rank of Professor and is the Director of Central Electron Microscope Unit. He was a consultant to the PAHO-WHO, was a Visiting Staff Scientist at the E.O. L. Berkeley National Laboratory, Berkeley, California, and a Visiting Scientist at the National Museum of Natural History in Paris, France.

SCIENTIFIC PAPERS WRITING AND RESEARCH ACTIVITIES
His research interests include ultrastructure of parasitic nematodes, especially the filariae and Trichinella, and the elucidation of the relationship of the Wolbachia endosymbionts with their filarial hosts. He has authored over 50 peer-reviewed publications, has been an invited speaker at local, national and international conferences and has organized the 4th International Wolbachia Conference in Puerto Rico in 2006.
Dr. Laura Kramer is currently Associate Professor of Veterinary Parasitology and Parasitic Diseases, University of Parma Veterinary School, Parma Italy

EXPERIENCES and RESEARCH ACTIVITIES
She began her experience in Veterinary Parasitology in 1996 as a study grant recipient for a project involving the local immune response to sarcoptic mange infection in swine. Her background in veterinary pathology and her experience with immunohistochemical/ultrastructural studies of dendritic cells in various animal species lead to an interest in host-parasite relationships and parasite immunology.

Starting in 1998, collaboration with colleagues at the University of Milan on canine and feline heartworm disease lead to the participation in several research projects (national and European) on the epidemiology and immune response to filarial infection and the role of bacterial endosymbionts in the biology of filarial nematodes.

Other research interests and activity include the epidemiology of canine neosporosis and the role of local immune responses to Leishmania infantum infection in dogs, arthropod pests in stored and aged food products.
Suzana Otašević (previously Suzana Tasić), Professor

PERSONAL DATA
Birth place: Niš, Serbia;
Full Professor of Microbiology and Parasitology, University of Niš, Serbia.

ACADEMIC AND ADMINISTRATIVE POSITIONS
Currently working as a professor responsible for the area of Medical parasitology and Medical mycology in the number of cases of integrated study of medicine, dentistry, pharmacy, and high professional studies in the primary, and post-graduate study programs at the University of Niš, Serbia. In the Public Health Institute Niš employed as specialist of Microbiology and Parasitology. Head of the Department of Parasitology and Mycology, Institute of Public Health Niš (1997-2004). Member of the Ethics Committee of the Medical Faculty, University of Niš. The vice-president of Serbian Society of Medical Mycologists, member of board of Society of Serbian Parasitologists.

SCHOLARSHIPS AND AWARDS
Winner of the prize for the best young scientists in Serbia granted by the Ministry of Science in 2000. Professional training in the field of medical parasitology, mycology and virology in reference laboratories in Belgrade: Republic Institute of Public Health, Department of Microbiology and immunology, University of Belgrade, Medical faculty; Institute for Immunology and Virology-Torlak.

TEACHING ACTIVITY
Teaching activity from 1989 to 2014 in Graduate, PhD and Master studies in the field of Parasitology and Mycology at the University of Niš.

SCIENTIFIC PAPERS WRITING AND RESEARCH ACTIVITIES
Current research topics: Parasitological and epidemiological investigation of vector-born zoonosis; Micological and epidemiological investigation of superficial mycoses. Participant of four five-years duration projects funded by the Ministry of Science of Serbia: Infective agents in medicine. Microbiological, histopathological and clinical research in medicine; Survey of some risk factors for the formation, maintenance, and spread of infectious process in the population of south-eastern Serbia; Risk factors and other epidemiological characteristics of suicide in South Serbia; The importance of laboratory evidence of early biomarkers for outcome of invasive fungal infections in our country. More than 70 articles, 4 books and more then 120 summary-publications from participation in scientific conferences at home and abroad. Reviewer: Journal of AIDS and HIV Research (JAHR); Facta Universitatis, Acta Fac Med Naiss, Acta medica mediana. Organizer and lecturer at numerous lectures of continuous medical education organized by Medical Faculty Nis, Institute of Public Health. Guest lecturer in the field of parasitology and mycology. Editor of textbook Medical parasitology-book with CD, author of a textbooks: 'Fungal infection, diagnosis and therapy, Oral microbiology, Medical mycology and parasitology.
Fernando Simón Martín, Professor

PERSONAL DATA
Birth place: Salamanca, Spain;
Full Professor of Parasitology, University of Salamanca (USAL), Spain.

ACADEMIC AND ADMINISTRATIVE POSITIONS
Current Head of the Department of Animal Biology, Parasitology, Ecology and Agricultural Chemistry; Secretary of the Department of Animal Biology and Parasitology (1987/88); Member of the Research Council of the USAL (2008/2012).

SCHOLARSHIPS AND AWARDS
Scholarship for movility of researchers (1991-1992) applied in the Laboratory of Immunology and Parasite Pathology of the University of Puerto Rico. Award “Golden Lancet” 2010 of the Russian Small Animal Veterinary Association (RSAVA), Moscow. Honorary Member of the Don Small Animal Veterinary Association (Russia).

TEACHING ACTIVITY
Teaching activity from 1979 to 2014 in Graduate, PhD and Master studies in the field of Parasitology at the USAL. Lectures in the Puerto Rico, Milan, Antioquia (Medellín) and Coimbra Universities. Exchange programs (recipient for graduates and students) from the Universities of Puerto Rico, Antioquia and Milán (funded by USA, EU and Spanish programs).

RESEARCH ACTIVITIES AND SCIENTIFIC PAPERS WRITING
Dr. Luigi Venco, DVM, SCPA, EVPC Dipl

PERSONAL DATA
Graduated at the Faculty of Veterinary Medicine of the University of Milan, obtained 2 years later the Board in Small Animal Practice in the same Faculty and attended the Course of Cardiology in dogs and cats at the University of Turin.

ACADEMIC AND ADMINISTRATIVE POSITIONS
He was as Visiting Professor for six months at Faculty of Veterinary Medicine of Georgia (Athens USA) and visited further several Veterinary Teaching Hospital abroad (Davis CA, Ft Collins CO, Philadelphia PA, Gifu Japan
Since 2006 he is a Diplomate of the EVPC (European Veterinary Parasitology College).
He works at the Veterinary Hospital “Città di Pavia” in the fields of Cardiology (both medicine and surgery) and Clinical Parasitology.

SCIENTIFIC PAPERS WRITING AND RESEARCH ACTIVITIES
He presented more than 100 topics in Italy, U.S.A., U.K., Spain, Slovenia, Greek, Poland, Croatia, Japan, Cuba, Czech Republic, Bulgaria, Serbia, Russia about Parasitology and Cardiology and is author and co-author of more than 20 papers on “Internationally peer reviewed journals”, two books about Heartworm disease, one book about Small Animal Parasitology and one about Cardiology of dogs, cat and horses.
He works in cooperation in research projects with the Faculties of Veterinary Medicine of Milan, Parma, Zagreb and the Faculty of Biology of Salamanca.
THURSDAY, 3 JULY, 2014 · 09:30-09:50  ● Invited Speaker

NEWS ABOUT HUMAN DIOFILARIOSIS

Fernando Simón1, Vladimir Kartashev2, Mar Siles-Lucas3, Rodrigo Morchón1, Javier González-Miguel1

1Laboratory of Parasitology, Faculty of Pharmacy and Institute of Biomedical Research of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain
2Department of Infectious Diseases, Rostov State Medical University, Rostov-na-Donu, Russia
3IRNASA, CSIC, Salamanca, Spain

Introduction: Dirofilariosis is a vector borne zoonotic disease mainly caused by Dirofilaria immitis and D. repens, filarioid species whose primary hosts are domestic and wild canines and felines. The adaptation Dirofilaria/man is incomplete. Therefore, human infections usually result in the early destruction of larvae without the appearance of signs revealing the presence of parasites, except the existence of specific antibodies in blood. However, in an unknown portion of infections immature worms can cause benign subcutaneous or pulmonary nodules or ocular locations. Suspicion of a malignant origin when nodules are detected as well as the surgical removal of the worms are responsible for the most significant damages associated to human dirofilariosis.

Discussion

From 2011 to 2014, about 103 papers related to human dirofilariosis have been published from which 80% corresponded to case reports and retrospective reviews of previously published cases, while other aspects received much less attention. Subcutaneous/ocular dirofilariosis is detected with a frequency 10 times higher than pulmonary dirofilariosis (approximately 3945/375 cases, respectively), being Ukraine, the Russian Federation (RF) and India the countries where the highest increase in reported cases has been recorded in the last 3 years. This increase is allowing the observation of unknown or poorly documented aspects of human dirofilariosis. Ocular location is detected with increasing frequency. 43.39% of 719 cases clinically documented in the Russian literature presented ocular location. Although the vast majority of ocular cases are attributed to D. repens, it has been recently observed that in 4 out 45 ocular cases molecularly characterized (ribosomal 12s and cox-I sequences), the causal agent was D. immitis. Two cases with retrocular location affecting the optical nerve have been reported in a short time in the RF. At least in two cases, live worms showed erratic movements around the eye, similar to Loa loa. An unusual case of a live D. immitis worm in the coronary arteries of a patient undergoing catheterism was described in Croatia. The steady increase of reported cases may show in the future if these are isolated facts or whether we should change the traditional pattern of human dirofilariosis.

Conclusions: From the experimental research point of view it has been observed that individuals with pulmonary dirofilariosis develop IgG antibodies against most of the isoforms of 4 proteins of D. immitis activating the fibrinolitic system (Actin, FBAL, GAPDH and GAL), which probably limits the capacity of the worms to prevent thromboembolisms in these individuals.
THURSDAY, 3 JULY, 2014 • 09:50-10:20 • Invited Speaker
FOCUS ON ESCCAP GUIDELINES FOR DIAGNOSIS, PREVENTION AND CONTROL OF DIROFILARIA INFECTION IN DOGS AND CATS
Claudio Genchi
Department of Veterinary Science and Public Health, University of Milan, Milan, Italy

Introduction
ESCCAP (European Scientific Counsel Companion Animal Parasites) is an independent, non-profit making organization consisting of experts in the field of parasitology and public health from across Europe. Twelve National Associations (Austria, Benelux, France, Germany, Italy, Denmark, Hungary, Poland, Portugal, Spain, Switzerland, UK and Ireland) are associated with ESCCAP Europe.

Discussion
ESCCAP's role is to develop guidelines for the treatment and control of parasites in pet animals protecting the health of pets, enhancing the safety of the public, and preserving the bond between pets and people.

The ESCCAP aim is to provide veterinary professionals with free expert information in the form of Guidelines derived from independent and soundly based investigation and research which are continually updated and revised.

Currently, 5 Guidelines on the control of worms (G1), superficial mycoses (G2), ectoparasites (G3), vector-borne diseases (G5) and intestinal protozoa (G6) of dogs and cats are available at www/esccap.org. The past recent years have seen a marked increase in the spread and range of pet parasite diseases throughout Europe.

Dirofilaria and other vector-borne infections have progressively spread from Southern Europe to Northern and Eastern regions previously considered not affected by such a parasites and now at risk of endemisation. For example, D. repens is well know to be endemic in Hungary at least from the last decades of 1900 and several human infections have been reported from 2000 on. Furthermore, recently the first case of D. immitis in a pet ferret (2009) followed by several cases in dogs (2011-2012) have been reported which suggest an active transmission of the parasite. In ESCCAP Guidelines, the prevention and control of Dirofilaria and other filarial infections are part of the Guideline 5, Insect-borne infectious agents and Tick-borne infectious agents of dogs and cats in Europe. Currently, the guideline briefly describes the biology, the transmission and the distribution of both D. immitis and D. repens further other filarial parasites such as Acanthocheilonema and Cercopithifilaria.

The clinical findings of Dirofilaria infection in both dogs and cats are described to address the veterinary practitioner in the suspicion diagnosis. The current information on diagnostic methods for differentiate microfilariae in the blood (Knott test), serology for detection of circulating D. immitis adult female antigens and other approach as radiography, electrocardiography and echocardiography are given and critically considered for both dogs and cats. The morphological features of microfilariae, including length, width, morphology of cephalic end tail and acid phosphatase stain are done.

The significance and the role played by Wolbachia, the obligate endosymbiotic bacterium of filarial worms, in the pathogenesis of the infection is briefly explained and the possible use of an antibiotic treatments to deplete them from the parasite is suggested to control/decrease
thromboembolic risks consequent to the inflammatory effect due to the released bacteria during treatment.

The risks and the advantages of the different protocols for the adulticidal therapy both in dogs and cats are discussed and clarified. The minimum-maximal doses of the current preventatives are given for dogs and cats. Control strategies are suggested either for animals living in or travelling in and from endemic areas.
THURSDAY, 3 JULY, 2014 • 10:20-10:35 ● Invited Speaker

KNOW YOUR (DIROFILARIA) ENEMY … AT THE ULTRASTRUCTURAL LEVEL

Wieslaw J. Kozek
Department of Microbiology and Medical Zoology and Central Electron Microscopy Unit, Medical Sciences Campus, University of Puerto Rico, San Juan, Puerto Rico.

Introduction
The two morphological forms of *Dirofilaria* seen in veterinary clinics are the microfilariae and the adults. Since the resolution of the light microscope is limited, these two forms were studied by transmission electron microscopy (TEM) to elucidate their ultrastructure and thus complement and amplify our information about them obtained by light microscopy. The adults and microfilariae of *Dirofilaria immitis*, obtained from dogs harboring naturally acquired infections, were processed by established standard procedures for examination by TEM.

Objectives and Discussion
Both forms have the same structural systems as the vertebrates: muscular, digestive, nervous, excretory, secretory, reproductive and (exo) skeletal, although some are modified or embryonal in the microfilaria. The body wall of microfilaria consists of multilayered cuticle, hypodermis and muscle cells oriented along the long axis of the larva. The digestive system consists of primordial esophageal tube and embryonic cells for future development of the intestine; internal lining of the anal vesicle consists of microvilli, suggesting an absorptive function. The reproductive systems are not developed. Nervous system consists of the nerve ring, peripheral nerves, amphids in the cephalic space and phasmids in the tail region. Excretory system consists of the excretory cell and its cytoplasmic appendage that terminated in the excretory vesicle. Adult worms have the same structural model as the microfilariae but all the system primordia have developed and matured, especially the digestive and the reproductive systems. The multilayered cuticle is covered with a lipid-rich trilaminar, hydrophobic epicuticle. Subcuticular, cylindrical hypodermis envelops the muscle cells and the perienteric cavity which contains perienteric fluid and houses the digestive and the reproductive systems; its internal surface is porous, enabling interchange of materials from the perienteric fluid. The amphids and cephalic papillae are more complex, the ganglia of the nerve cells are located above and below the nerve ring. Digestive system consists of short buccal capsule, the esophagus, intestine composed of columnar epithelial cells and the rectum. The ovaries are didelphic and contain a branched rachis; posterior end of each ovary contains the primordial germinal cells which produce the primary oogonia that develop and mature during their passage to the seminal receptacle, where the oocytes are fertilized. The uteri have external muscle cells, contraction of which propels uterine contents anteriorly. The sperm cells are amoeboid. *Wolbachia* are found in the hypodermal chords in both sexes and in germinal cells of the female, in developing oocytes, and microfilariae. The structure of *Dirofilaria* indicates an extraordinary adaptation to its node of existence and the limited number of its chemotherapeutic targets that it offers will continue to present challenges to current and future control measures.

*Dirofilaria immitis* has a didelphic, approximately 9 cm-long ovary. The posterior tip of the ovary (approx. 16 x 48 µm) is dilated, similar to a head of a match stick, without any evidence of a cap cell. Subsequent portions of the ovary can be divided into the germinative zone (approx. 2.5 cm), followed by a maturation zone (approx. 2 cm), and the...
longest portion of the ovary, the growth zone (approx. 4 cm), where the oocytes increase in size and mature before entering the seminal receptacle through a 3-5 mm long oviduct.

In many nematodes, including some filariae, the rachis is a cytoplasmic trunk to which oocytes are attached and which is believed to be the source of Wolbachia for the ooocytes. However, in *D. immitis*, the rachis is a delicate cytoplasmic reticulum that extends into the germinative zone, apparently contacting each oocyte, but these processes lack Wolbachia. The primary germinal cells and the primary oocytes were heavily infected with Wolbachia, had scant ribosomes, and apparently lacked mitochondria. This contrasted to the scarcity of Wolbachia and proliferation of mitochondria seen in the mature oocytes.

**Conclusions**

These observations suggest that:

(i) in *D. immitis*, vertical transmission of Wolbachia is principally from the primordial germinal cells; the importance of transmission from the rachis may be secondary and insignificant;

(ii) Wolbachia in primary germ cells should be the principal target for chemotherapy and (iii) the function of Wolbachia may change as the oocytes mature. It is possible that, in the germ cells and in the primary oocytes, Wolbachia may provide the initial metabolic energy needs, similar to that of mitochondria, which may be later replaced by mitochondria when these proliferate in more mature stages of the oocytes.
Despite the name “Heartworm” suggests a primitive cardiac involvement as the main localization of the worms and the first damages are in pulmonary arteries, heartworm disease should be considered a pulmonary disease that in the last stage only may involve right cardiac chambers.

In Heartworm infected dogs the reduction of compliance and gauge of pulmonary arteries, that can be also occluded by either thromboembolism or severe villous proliferation, results in a hypertensive pulmonary state and, as a consequence, in an increased after load for the right ventricle which can induce “cor pulmonare” and right cardiac congestive heart failure (Rawlings, 1986; Calvert et al., 1988).

Based on the pathogenesis the clinical evolution of heartworm disease in dogs is usually chronic.

Most infected dogs do not shows any symptoms of the disease for a long time, months or years, depending on worm burden, individual reactivity and exercise, as arterial damages are more severe in dogs with intensive exercise than in dogs at rest. Signs of the disease develop gradually and may begin with a chronic cough.

Together with antigen with history, clinical examination and antigen testing thoracic radiographs and echocardiography are pivotal for staging the disease and choosing the best therapy in infected dogs.

**Thoracic radiographs**

Thoracic radiographs may show, in the advanced stage, enlargement of the pulmonary arteries, abnormal pulmonary patterns and in the worst cases right sided cardiomegaly. If congestive right heart failure is present peritoneal and pleural effusion can be noted. They are useful to assess the severity of the pulmonary lesions but not for evaluating worm burden (Venco et al., 2004). Since radiographic signs of advanced pulmonary vascular disease may persist long after an infection has run its course, some of the most severely diseased dogs may have disproportionately low worm burden. On the contrary some inactive dogs may have large worm burdens and be clinically asymptomatic with no or trivial radiographic lesions.

**Echocardiography**

Echocardiography allows a direct visualization of cardiac chambers and connected vessels (Moise, 1988).

It also allows the visualization of parasites in right cardiac chambers, caudal vena cava, main pulmonary artery and proximal tract of both caudal pulmonary arteries. The heartworms are visualized as double, linear parallel objects floating in the right cardiac chambers or into the lumen of vessels (Moise, 1988; Badertscher et al., 1988).

It is performed mainly in cases where clinical and radiographic findings suggest severe disease.

Cardiac ultrasound can increase the accuracy in staging the disease and estimating the worm burden, both of which affect the treatment program and the prognosis (Venco et al., 2003). Doppler echocardiography by measuring the velocity of tricuspidal and or pulmonic valve...
regurgitation furthermore allows a not invasive correct assessment of pulmonary pressure.

**THERAPY**

It has been said that the treatment of heartworm infection is difficult. There are several strategies that can be used including the option of not treating at all. The important concept to realize is that treating for heartworm infection is neither simple nor safe in itself.

Prior to therapy, the heartworm patient is assessed and rated for risk into one two categories. The most important factors that need to be evaluated are:

- Worm burden (based upon the ELISA tests performed and ultrasound examination) (Venco et al., 2004)
- Severity of pulmonary vascular disease (based on thoracic radiographs and echocardiography) and presence of pulmonary parenchymal abnormalities (thoracic radiographs).

Others important factors include: concurrent health factors, degree to which exercise can be restricted in the recovery period, and owners compliance.

The categories into which patients are grouped are as follows:

**Low risk of thromboembolic complications** (low worm burden and no parenchymal and/or pulmonary vascular lesions)

Dogs included in this group must satisfy all these conditions

1) No symptoms  
2) Normal thoracic radiographs  
3) Low level of circulating antigens or a negative antigen test with circulating microfilariae  
4) No worms visualized by echocardiography  
5) No concurrent diseases  
6) Permission of exercise restriction

**High risk of thromboembolic complications**

In this group should be included all dogs that don’t satisfy one or more of these conditions

1) Symptoms related to the disease (coughing, lipotimias, swelling of the abdomen)  
2) Abnormal thoracic radiographs  
3) High level of circulating antigens  
4) Worms visualized by echocardiography  
5) Concurrent diseases  
6) No permission of exercise restriction

Symptomatic therapy include drugs and measures that can improve cardiopulmonary circulation and lung inflation in order to relief symptoms in dogs that can not undergo causal therapy or to prepare them for a adulticide or surgical therapy.

Restriction of exercise and in selected cases cage rest seems to be the most important measure to improve cardiopulmonary circulation and reduce pulmonary hypertension (Dillon et al., 1995).

Anti-inflammatory doses of glucocorticosteroid (prednisolone 2 mg / kg s.i.d. for four or five days) given at diminishing rate can control pulmonary inflammation and thromboembolism. Diuretics (furosemide 1 mg / kg b.i.d.) are useful when right congestive heart failure is
present to reduce fluid effusions. Digoxin may be administered only to control atrial fibrillation. The use of aspirin is debatable and as not secure proofs of beneficial antithrombotic effect have been reported, for this reason the empiric use of aspirin is not advised since long time (Knigh, 1995).

The organical arsenical compound melarsomine dihydrochloride is actually the only used and effective heartworm adulticide.

Two intramuscular injections of 2.5 mg / kg 24 hours apart is the standard regimen but dogs to reduce the risk of pulmonary thromboembolism a more gradual two step treatment is strongly advised by giving just one injection and then administering the standard pair of injections at least 50 days later (Keister et al., 1992; Rawlings et al., 1996). In fact one administration of melarsomine at the dose of 2.5 mg / kg kills about 90 % of male worms and 10 % of female worms resulting therefore in 50 % reduction of the worm burden (which is safer in terms of embolism and shock). For this reason the three-injection alternative protocol is the treatment of choice of the American Heartworm Society and several university teaching hospitals, regardless of stage of disease.

Pulmonary thromboembolism is an inevitable consequence of a successful adulticide therapy. If several worms die widespread pulmonary thrombosis frequently develops. Mild thromboembolism may be clinically unapparent but in severe cases life threaten respiratory distress can occur. These complication can be reduced by restriction of exercise (no walks, no running around. The dog must live the indoor life or, in selected cases a cage rest) during the 30-40 days following the treatment and by administration of calcium heparin and anti-inflammatory doses of glucocorticosteroid to control clinical signs of thromboembolism (Di Sacco et al., 1992; Vezzoni et al., 1992; Rawlings et al., 1996).

It is now known that certain macrolides have adulticidal properties (McCall et al., 2001). Experimental studies have shown ivermectin to have partial adulticidal properties when used continuously for 16 months at preventative doses (6-12 mcg/kg/month) and 100% adulticidal efficacy if administered continuously for over 30 months (McCall et al., 2001). While there may be a role for this therapeutic strategy in very few and selected cases in which patient age, financial constraints or concurrent medical problems prohibit melarsomine therapy, the current recommendations are that ivermectin not is not adapted as the primary adulticidal approach, and that this kind of therapy should be used carefully. In fact the adulticide effect of ivermectin generally requires too long before heartworms are eliminated completely. The older the worms when first exposed to ivermectin, the slower they are to die. In the meantime, the infection persists and continues to cause disease. Clinical observations suggest that heartworm-positive active dogs in prolonged ivermectin treatment may worsen if ivermectin is given monthly for 2 years (Venco et al., 2004). Adding a course of Doxycycline (10 mg/Kg once a day for 30 days, oral route) that cleans the Heartworms of the endosymbiont Wolbachia sp. and giving ivermectin twice in a month leads to a more effective and safe therapy

Surgical therapy is advised when several worms displacement in the right cardiac chambers produces the sudden onset of severe symptoms (caval syndrome). It can be accomplished under general anaesthesia with Flexible Alligator Forceps introduced via jugular vein. Flexible Alligator Forceps aided by fluoroscopic guidance can access not only right cardiac chambers but also pulmonary arteries. The main pulmonary artery and lobar branches can be accessed with flexible alligator forceps,
aided by fluoroscopic guidance (Ishihara et al 1990). Intra-operative mortality with this technique is very low. Overall survival and rate of recovery by dogs at high risk of pulmonary thromboembolism is improved significantly by physically removing as many worms as possible. When the facilities are available, worm extraction is the procedure of choice for the most heavily infected and high risk dogs. Before electing this method of treatment, echocardiographic visualization of the pulmonary arteries should be performed to determine that a sufficient number of worms are in accessible locations.

Surgical removal of heartworm can avoid pulmonary thromboembolism, as compared to pharmacologic adulticides, such as melarsomine (Morini et al., 1998). This procedure, however, requires specialized training and instrumentation, including fluoroscopic imaging capabilities. Nevertheless, it remains a very good and a safe alternative for the management of high risk patients and the best choice in dogs harbouring a large worm burden.

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THURSDAY, 3 JULY, 2014 · 12:10-12:40 ● Invited Speaker

IMPORTANCE OF MICROFILARIAL CONTROL AND OPTIONS FOR REMOVAL WITH 10% IMIDACLOPRID + 2,5% MOXIDECTIN

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Introduction

In the United States, heartworm isolates have now developed in dogs on preventive therapy, and there are microfilariae not cleared by the administration of monthly preventives after adult heartworm treatments with melarsomine dihydrochloride. Clearing microfilariae from the blood of dogs should minimize the threat of spreading resistant isolates to new areas or allowing it to expand its hold in areas where already present.

Objectives: A report will be given on the status of heartworm resistance and the value of utilizing Advocate (Advantage Multi) for Dogs to minimize the transmission of resistant isolates.

Materials and Methods

The presentation will review heartworm resistance in the United States relative to the macrocyclic lactones, i.e., ivermectin, milbemycin oxime, selamectin, moxidectin slow-release injectable, and topical moxidectin. It will also present laboratory studies and clinical field-trials on the effectiveness of Advocate in the clearance of microfilariae from heartworm infected dogs with or without concomitant adulticide treatment with melarsomine dihydrochloride.

Results

Heartworm products now in the United States have now been shown to fail against one or several heartworm isolates when tested using the same methodology by which they were originally tested and found to be 100% protective. Elevated doses of ivermectin and milbemycin oxime have also failed to clear dogs of their circulating microfilariae even when given at elevated dosages or with increased frequency. Advocate testes at six field sites had an overall microfilarial clearance rate of greater than >99% whether or not the dogs were concomitantly treated with melarsomine dihydrochloride. Out of these six sites, there was one where microfilarial clearance was<90%.

Discussion

There are isolates in the United States which have been demonstrated to not be 100% protective against heartworms when tested as originally for FDA approval as heartworm preventives. Also, these isolates give birth to microfilariae that with stand increasing quantities of macrocyclic lactones. Advocate was highly efficacious against circulating microfilariae, but the reduced efficacy at the one field site may be an indication that resistant isolates are more common in some portions of the United States than others.

Conclusions

Advocate has fared better than the other marketed preventives in comparison trials against several of the resistant isolates, and the utilization of Advocate to clear dogs of their microfilariae should provide a major aid in stemming the spread of resistance. However, veterinarians need to be cognizant of the risk of resistance expanding within some areas or being introduced by traveling dogs into new areas.
Introduction: After the first case of human subcutaneous dirofilariosis (HSD) described in the former Soviet Union in 1915 only a few cases were reported over the next decades. In the late 90s the number of HSD patients was significantly increased resulting in a growing interest of clinicians and epidemiologists. Objectives: The purpose of this study was to evaluate the dynamics of incidence and geographical distribution and extrapolation of the obtained data to the climate change.

Results and discussion
Requests to territorial epidemiology services of all 83 subjects of the Russian Federation revealed that dirofilariasis was registered in 1997-2013 at the territory of 58 of them and the number of patients in this period amounted 1192. The highest morbidity was reported from Rostov region (222 cases) that borders with Donetsk and Lugansk regions of Ukraine where the HSD incidence is also high. Morbidity was growing in Nizhny Novgorod (129 cases), Volgograd (93), Saratov (58), Astrakhan (50) regions and in Moscow city (54). Ukrainian government legally introduced notification all HSD patients in 1997. During the 1997-2013 in Ukraine 1866 HSD cases have been identified. Data on the first 10 cases of HSD were published in Belarus in 2004. In 2013 the total number of registered HSD cases reached 80. Morbidity peaked in 2012-2013, during those two years 44% of all HSD cases in Belarus were identified. From 1997 to 2013 only 20 HSD patients have been identified in the Republic of Kazakhstan. It was clearly noticed a trend of increasing HSD incidence in the northern territories of the former USSR and slowing the dynamics of newly detected cases in the southern regions together with a significant shift of the boundary of the disease to the North. We found a correlation between the number of annual predicted generations of *Dirofilaria* with the rising temperature in the same time period of meteorological observations and the number of HSD case over the past 1997-2013 in each territory.

Conclusions
A prediction of the situation for 2030 based on the dynamics of case detection and the temperatures foreseen by the Russian Committee of Hydrometeorology was performed. At the current rate it is projected a 3-5 fold increase in the incidence and spread of the disease further to the North especially pronounced in the European part of the former USSR. To solve the problem of HSD control an integrated approach and coordination of activity with the veterinary service is required.
THURSDAY, 3 JULY, 2014 ∙ 13:10-13:40 ● Invited Speaker

CANINE AND HUMAN DIROFILARIA INFECTIONS: WHAT IS NEW IN THE BALKAN PENINSULA

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Introduction

Although dirofilariosis has long been known to veterinary practitioners and first data about heart canine infection date back to more than a century, this zoonosis is still a current problem worldwide. In spite of available diagnostic methods, effective prevention, possible treatments and increased attention in veterinary and now medical practice, canine and human dirofilariosis continue to be diagnosed and reported in many countries1, 2, 3. Dirofilarioses are vector-borne diseases, transmitted by mosquito of genus Aedes, Anopheles and Culex1, 2, 4, that usually affect domestic and wild carnivores. Humans are accidental hosts and infections have been most frequently detected as subcutaneous or subconjunctival; internal locations of the worms, as pulmonary, are rarely detected and scarcely identified. Dirofilaria immitis and Dirofilaria repens are the most frequently identified species in Europe3, almost all countries of the Balkan Peninsula included. We report here current data about the epidemiological scenario of canine and human infections in each of these Countries.

Discussion

In Greece three different filarioid nematodes (D. immitis, D. repens, and Dirofilaria grassii, now Cercopithifilaria grassii) were determined in dogs, with infection rates ranging from 12% to 37%, so that the country is recognized as endemic. Notwithstanding this datum dirofilariosis show low incidence in people5, 6; beside 40 cases of D. repens human infections described until 1997, in last years 3 more cases (2 by D. repens subcutaneous infection and 1 by D. immitis pulmonary dirofilariosis) have been reported in the country7, 8, 9.

In the territory of Turkey dirofilarioses are widely spread showing 1-27% prevalence, and in the region of Kirikkale infected dogs even reach 58%10. Literature also reports an uncommon human case of intravitreal Dirofilaria location11.

As for the Bulgarian territory, at the beginning of the new millennium first reports evidenced canine dirofilariosis in 1.4% of pets and in 12.5% of stray dogs. According to Kirkova12, D. immitis not only is a not-rare parasite in dogs, but it is also present in foxes and jackals, what can explain the infection spreading from wild canides (as natural reservoirs and source) to dogs. In 2014 all cases of human dirofilariosis documented in 39 years were collected and described, so that 47 cases were published or registered, showing an increasing incidence from 0.001 to 0.040 per 10^5 inhabitants; eighteen regions are endemic and 10 potentially endemic. Location of the parasite found was: subcutaneous tissue of different parts of the body (74.5%), subconjunctiva (21.3%), and genital organs -namely scrotum and testicles-(4.2%)13.

First data concerning Romania came from the 1930ties: few cases of dirofilariosis in dogs14, 15. By the end of XX century the epizootic situation changed, and very high prevalence of infected dogs was detected16; in following years it was announced that Western and South-Western territories of Romania are endemic areas for this canine parasitic infection17, 18.

Latest studies indicate a significant presence of various vector-born parasitic infections,
dirofilarioses included, in local dogs and in subjects imported in Germany from Romania 19. In spite of this epizootological scenario, in Romania only few cases of human dirofilariosis have been described, suggesting that the infection is probably more frequent than it appears 20. In Albania the first investigation was carried out in 1995-1996, and it was found that the coastal Western area of the country is endemic for dog dirofilariosis, as its prevalence is 13.5% 21. Further analyses showed that in the district of Tirana and in the area bordering Kosovo prevalence were 3% and 7%, respectively, without data about human cases of infection 22.

As for the territory of the former Yugoslavia, there are few data from Slovenia, and no reports of canine and human dirofilariosis are available from Montenegro, except two human cases which have been diagnosed in Serbia 23, 24. Nevertheless, it can be pointed out that in some countries, where in the past only sporadic cases of dirofilariosis due to D. repens and D. immitis had been found 25, the two parasites are now observed with increasing frequency. In detail, even if in referent literature we did not find data about dirofilariosis in Bosnia and Herzegovina, thanks to the kindness of our colleagues (Prof. dr Almedina Zuko and dr Amir Zahirović from Veterinary faculty of Sarajevo, B&H) who gave information in personal contacts, we can say that also this territory is endemic for dog dirofilariosis. The first conducted survey confirmed the presence of Dirofilaria spp. in 5.02% of the examined dogs, with a higher number of subjects positive to D. immitis (3.11%) than to D. repens (1.91%).

From the first reported case of dirofilariosis by Ježić and Simić in 1929 15 until today, there are only few data about dirofilariosis in the Republic of Macedonia, notwithstanding it is a country having suitable climatic and geographical characteristics, and possibly competent vectors like in neighbouring countries. Indeed, in 5-years period only 12 cases of D. immitis infected dogs were found at the necropsy; as for D. repens, studies including small numbers of dogs found infection rates ranging from 12.5% to 21.1%, and only two human cases (one subcutaneous and one subconjunctival) have been reported 26.

In the 1990ties in Croatia canine dirofilariosis was sporadically found and infections had been considered non-autochthonous 14. However, during the following years this parasitosis was evidenced with increasing frequency 27, and even human dirofilariosis due to D. repens has been described (12 cases, whose 4 ocular and 8 subcutaneous) 28.

After first systematic/methodic survey carried out in the territory of Serbia, we can point out that our country is endemic for dirofilariosis, whereas some areas as Voyvodina (Northern Serbia) are hyperendemic for D. repens infection in dogs 29, 30. The global warming and the increased traveling with pets on vacation may have affected the distribution and prevalence of arthropod-borne infections, and can explain the spreading of dirofilarioses to the Northern part of the country, considering that in the 1930ties Southern Serbia was hyperendemic (and now endemic) for this canine infection 31, 32, 33. According to Lazri 22, global prevalence of dog dirofilariosis in our Southern province of Kosovo is 9% (6.6% near Albania, and up to 16.1% in some different areas).

Considering that the epizootic situation of dirofilarioses in dogs and the presence of feeder-opportunistic mosquito species correlates with the occurrence of human infection, as demonstrated by the seroreactivity to Dirofilaria antigens evidenced in the examined population (5.3-27.1%) 34 we could expect many cases of human dirofilariosis. However, from 1971 till 2009 only 28 cases of human infection by D. repens have been described and published 24, surely less than expected. Worthily, in last years the awareness of this infection among physician is significantly improved in Serbia. In Belgrade 4 cases were detected in period of four last months (information was given, in personal contact, by Prof. Džamić). During the period from 2011-2014 in the Southern Serbia 5 cases of human dirofilariosis have been suspected and confirmed. In all cases the etiological agent was a D. repens female.
specimen, identified by parasitological and molecular analyses\textsuperscript{2, 36, 37}. In 4 patients the worm was found in subcutaneous tissues, whereas in one the filarioid was removed from the conjunctiva. This last case of subconjunctival infection was diagnosed in a patient living in the territory of Niš city, without anamnestic data about travels in endemic areas nor outside the city. Considering that a survey carried out in the city of Niš\textsuperscript{38} indicated this territory as maybe *Dirofilaria*-free, the case of human infection here acquired is quite surprising and suggests deeper investigations in the territory.

**As a summary**, we can conclude that the Balkan Peninsula is: i) mostly endemic for dog dirofilariosis; ii) human infections have been diagnosed mostly in recent years; iii) regions and countries never interested before to this zoonosis started researches and produced interesting data; iv) as a practical application of the concept of “one health”, more attention to this parasitic infection is paid not only from veterinary practitioners but also from physicians.

**References**


THURSDAY, 3 JULY, 2014 ∙ 14:40-14:55

EPIDEMIOLOGICAL VIEW ABOUT DIROFILARIOSES IN DOGS OF HUNGARY

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Introduction

Seven years ago, on the First European Dirofilaria Days (FEDD), in Zagreb it was shown the preliminary data of a survey performed 2005-2006, in Hungary (Fok et al., 2007). At that time the prevalence of *Dirofilaria repens* was 14% in dogs (116/826) and 6.9% in cats (2/29). This investigation was continued partly in the frame of PhD study.

The aim was to make a country-wide survey by collecting more blood samples from different regions.

Materials and methods

During the study 3104 blood samples of dogs were examined by modified Knott’s method. 2260 data sheets were filled out by local veterinarians and dog owners. 143 blood samples were checked by *D. repens* species-specific PCR, 24 samples by *D. immitis* species-specific PCR, and 7 samples by *Acanthocheilonema reconditum* species-specific PCR. Moreover the DNA of 10 samples were sequenced. We also made serological examinations from 125 blood samples by immunochromatographic Speed® Diro test (BVT, France). Fisher one-sided test and binomial one-sided test were used for statistical analysis of the data sheets. The common influence of the covariates was tested by probit regression.

Results

It was found 563 of 3104 blood samples of dogs microfilaria positive by modified Knott’s method, this means 18.1% prevalence. In certain areas the percentages were higher than 30%. According to morphological criterions 561 were identified as *D. repens* infection, while 2 samples contained smaller microfilariae, suspected *A. reconditum*. *D. immitis* serological tests were negatives in case of 124 samples, and in one occasion it resulted doubtful positive line. *D. repens* species-specific PCR was positive in 87 of 143 cases. From the 56 PCR-negative samples 32 were microfilaraemic by modified Knott’s method. *A. reconditum* species-specific PCR was negative in all 7 cases. Using *D. immitis* specific primer 1 sample proved to be positive. The same sample contained *D. repens* DNA too. This coinfection was originated in Hungary. The statistical analysis of the results showed, that *D. repens* infection is more frequent in dogs which are older, male, living in extensive circumstances, mainly without antiparasitic treatment. Visiting or living at the coastal areas of rivers seems to be a significant risk factor to acquire the infection.

Conclusions This prevalence was much higher than it has been found earlier. These data show that the rate of infection in Hungary is close to that of the Mediterranean countries. Moreover the study highlighted the possible zoonotic risks for humans living in the regions where the positive animals were found.

This study was supported by PhD scholarship (2006-2009) and Pfizer Animal Health (presently Zoetis Hungary), Budapest.
Introduction
Prevalence of *Dirofilaria repens* is well documented in Hungary since more than a decade. The first autochthonous cases of *D. immitis* appeared in the past few years. Prevalence of both parasites is on the rise however no exact prevalence data are available for *D. immitis* in Hungary.

Materials and Methods
In our laboratory we document presence of microfilaraemia on routine blood film examinations. Furthermore Knott’s-test, *D. immitis* antigen ELISA (Canine heartworm SNAP, IDEXX, USA) and *Dirofilaria* six species PCR tests are available in our laboratory or in cooperation with international diagnostic companies.

Results
Overall prevalence of microfilaraemia on routine blood films (n=21887) evaluated between 2009-2013 was 5.6%. Microfilaraemia showed a seasonal prevalence with minimum values during the winter months (1.16% in February) and maximum the summer months (10.4% in July).

Overall seroprevalence for *D. immitis* antigen was 16.5% in 260 tests performed during the observed period. 43.5% of these samples had no microfilaraemia on routine blood film examination whereas 27.5% were microfilaria positive.

Out of 33 „six species *Dirofilaria* PCR” test carried out during the given period 11 (33%) were positive for *D. immitis* with the same result for *D. repens*. Six (18%) samples were identified to carry both *D. repens* and immitis DNA. Five samples (15%) were negative for all six *Dirofilaria* species tested.

Conclusion
Our results supplement the prevalence data available for dirofilarioses in Hungary. We believe that our *D. immitis* sero- and PCR because positivity results overestimate the true prevalence of this parasite for several reasons, mostly because of biased patient selection. However no large scale seroprevalence studies are available for Hungary so far, albeit the knowledge of (at least near) true prevalence of this disease would aid interpreting serological results obtained by any method.
Introduction

Dirofilaria (Nochtiella) repens is a habitual parasite of dogs, cats and other carnivores. Most frequently, it lives in the subcutaneous tissue of its host. The intermediate hosts and the vectors are mosquitoes of the family Culicidae (Aedes, Culex and Anopheles). In the temperate regions of Europe, mainly Italy, France, Greece, and in recent years Hungary D. repens has occasionally been contracted, causing dirofilariosis in humans.

Objectives: The aim of this presentation is report of human D. repens infection diagnosed in National Center for Epidemiology (Budapest, Hungary) in last 13 years.

Methods

The parasitological diagnosis is based on the morphology of the extracted intact worm (after surgical extraction of the worm) and/or specific histopathological findings. The Knott concentration technique was used for detection of microfilariae in blood samples.

Results

From 2001-2013, at the National Center for Epidemiology 88 dirofilariosis cases were diagnosed. The average age of the 88 patients was 46,5 years. Of the 88 cases 34 showed ocular, 53 subcutaneous localization, and 1 was diagnosed in the histopathological section of a removed lymph-node in a patient with lymphoid leukaemia. To detect microfilariae the Knott concentration technique was used in 47 cases, with 1 positive result. Based on the available epidemiological data, it can be concluded that most of these cases are autochthon infections. Analyses of the territorial distribution of these 88 cases showed that they were localized on the watershed of the Danube or Tisza River, and in one case in close proximity to Lake Balaton.

Discussion and conclusions

During the recent years, Europe has experienced the spreading of D. repens infection from the “classical” Mediterranean countries toward the northern and eastern ones. Climate change seems to be the key factor responsible for more northerly distributions of the vectors and their possibility to transmit pathogens. Travel, trade, and insecticide resistance have also facilitated these processes. In conclusion, the transmission and spread of Dirofilaria species are influenced by multiple factors and depend especially on climatic conditions, host and vector populations. Visiting or living near riverbanks, where mosquitoes are abundant, appears to be a significant risk factor in contracting the infection. Since the detection of dirofilariosis needs improved clinical diagnosis, the increasing number of cases suggests that direct attention must be paid to this zoonosis.
CANINE DIROFILARIOSIS IN SLOVAKIA – THE RESULTS OF 8-YEAR EPIDEMIOLOGICAL RESEARCH
Martina Miterpáková, Adriana Iglódyová, Daniela Antolová, Zuzana Hurníková
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Introduction
The first systematic research on canine dirofilariosis in Slovakia started in 2007, two years after the first record of *Dirofilaria repens* in Slovak dog.

Materials and Methods
The samples collection was engaged in cooperation with veterinary practitioners from all regions of Slovakia. Dogs of various breeds, utilisation, age and gender were chosen randomly. Microfilariae in blood were detected by means of modified Knott test. DNA from positive samples was extracted and PCR analyses were provided for amplification of the CO1 gene fragments using specific *D. repens*, *D. immitis* and *A. reconditum* pair of primers.

Results
Between 2007 and 2013, more than 3,000 dogs from all regions of Slovakia were examined. Microfilariae were detected in 390 individuals with an overall prevalence of 12.1 %. Molecular analyses confirmed *D. repens* in all infected dogs. Co-infection with *D. immitis* was detected in 9 dogs from southern territory of Slovakia. None dog have been infected with *A. reconditum*. Great regional differences of *Dirofilaria* prevalence were observed with the highest occurrence of the parasite in south-western part of the country (Nitra and Trnava regions, prevalence 26.3 % and 20.5 %, respectively). In northern Slovakia the prevalence of canine dirofilariosis varied between 2.1 % and 5.4 % in individual regions. Microfilariae were found more often in the blood of guard and hunting dogs (prevalence over 20.0 %) than in companion dogs (mean prevalence of 3.9 %). The infection was more frequently detected in giant and large breeds, and also in shorthaired and wirehaired individuals (13.0 % and 15.1 %, respectively) as compared with longhaired dogs (6.2 %). The number of microfilariae in infected dogs varied from 1 to more than 4,000 per 100 µl of blood with the highest mean value in May and August and with the lowest numbers in February.

Conclusion
The results of the first research on *Dirofilaria* demonstrate that *D. repens* is widespread among dogs in southern parts of Slovakia characterised by specific environmental conditions with high mean annual air temperatures and periodic floods in the river system. Due to the zoonotic character of *D. repens*, in Slovakia 6 human cases of infection were officially registered between 2007 and 2013.

This study was supported by Science Grant Agency VEGA No. 2/0011/12.
FILARIOID INFECTIONS IN DOGS FROM ROMANIA: A BROADER VIEW
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Introduction
During the last decades, Dirofilaria spp. infection in European dogs has rapidly spread from historically endemic areas towards Eastern and North-Eastern regions. At the moment, little or no epidemiological data regarding these nematodes are available from such countries. So far, studies conducted in Romania were focused on the western districts and Bucharest area, so the objective of the present study was to provide a larger view, by assessing the prevalence and diversity of filarioid species infecting dogs from various areas of the country.

Materials and Methods
Between July 2010 and March 2011, blood samples were collected from 390 dogs from 23 localities, situated in 9 counties of Romania. Serological SNAP tests were performed for the detection of Dirofilaria immitis antigen. The remaining blood clots were stored at -20°C and later used for DNA extraction followed by multiplex PCR for assessing filarioid species diversity (Dirofilaria immitis, D. repens and Acanthocheilonema reconditum) using previously published primers.

Results
The molecular prevalence for each species was as follows: 6.92% (n=27) for D. repens, 6.15% (n=24) for D. immitis and 2.05% (n=8) for A. reconditum. In 45.83% (n=11) of dogs positive for D. immitis, coinfections with D. repens were observed. SNAP tests showed a slightly higher prevalence for D. immitis (7.17%, n=28). However, only 53.57% (n=15) of those antigen positive samples were confirmed by PCR, while other dogs (n=9) that were PCR-positive for D. immitis were negative in serology. The local prevalence for each species varies within large limits: from 0% in Transylvania up to 50% in the Danube Delta, most likely in relation with local climate and abundance of mosquito vectors. Regarding inconsistencies between serological tests and PCR, in case of antigen positive and PCR negative samples the microfilariae may have been absent from the peripheral blood at the moment of sampling. Antigen negative and PCR positive samples can be explained by a low adult worm burden, in which case the antigen concentration is below the test’s sensitivity. Since molecular methods offer the possibility to identify more filarioid species, we consider them a more useful tool, keeping in mind also the zoonotic character of D. repens.

Conclusion
The present study shows that in Romania Dirofilaria species are largely present in the south and south-east of the country and D. repens seems to be the most common canine filarioid species.
EPIDEMIOLOGICALLY STUDY ABOUT NATURAL INFESTATION WITH DIROFILARIA IN SHELTERS LOCATED IN THE SOUTHERN PART OF ROMANIA.

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Introduction
Canine dirofilariosis is a parasitic disease produced by nematodes of the genus Dirofilaria. *Dirofilaria immitis*, the causative agent of heartworm (HW) disease is an intravascular parasite that resides in the pulmonary arteries and less often on the right side of the heart and vena cavae, harming important systems such as respiratory, cardiovascular, lymphatic and renal, and *Dirofilaria repens*, the causative agent of canine and feline subcutaneous dirofilariosis. Both are transmitted by infected mosquitoes, which by feeding themselves with blood from a healthy dog, insert third-stage larvae (L₃) in the dog’s subcutaneous tissues.

Objective
The aim of this study was to assess the prevalence of the dirofilariosis in dogs from shelters in the Southern part of Romania.

Material and Methods
The investigation has been carried out from September 2013 to February 2014. A total of 150 dogs has been enrolled, with ages between 1 and 10 years-old, all of them stray dogs and without any clinical signs. Non-coagulated blood samples were taken from each patient, in order to detect *D. immitis* or *D. repens* infestation. The presence of microfilariae in the blood samples was assessed by fresh blood smear, modified Knott’s technique (for the species identification of circulating microfilariae), and the serum was analysed for *D. immitis* antigen using SNAP*4Dx*Plus kit (IDEXX commercial product) with no cross-reactivity to *D. repens* antigens.

Results
Out of 150 enrolled dogs, 76 (50.67%) have turned out positive for circulating microfilariae. 62 out of the 150 studied (41.33%) were positive for circulating *D. immitis* microfilariae, 6 out of 150 (4%) were positive for circulating *D. repens* microfilariae, and 8 out of 150 (5.33%) were positive for both circulating *D. immitis* and *D. repens* microfilariae. Tested dogs were divided into different categories depending on age class (<2, 2-5, >5 years-old). Among infected cases, two animals (2.63%) out of 76 were under 2 years, 50 out of 76 (65.78%) were between 2-5 years-old and just 24 out of 76 (31.57%) between 5-10 years-old. A higher prevalence was recorded in the cases of 2 to 5 years-old dogs.

Conclusions
This study proves that canine dirofilariosis is spreading with the presence of both *D. immitis* and *D. repens* in shelters situated in the Southern part of Romania, and highlights the potential risk of healthy animals getting infected and spreading it to humans that live among them.
THURSDAY, 3 JULY, 2014 · 16:10-16:25
EPIDEMIOLOGY OF DIROFILARIOsis IN DOGS IN GREECE: PREVIOUS AND L ATEST INFORMATION
Anastasia Diakou, Emmanouil Kapantaidakis
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Introduction
Greece is endemic for canine dirofilariosis caused by *Dirofilaria immitis*. Most of the epidemiological surveys to date have been conducted in the northern country, where prevalence is reported up to 34.13%. Published and unpublished information about the southern country consent, that heartworm infection cases are sporadic.

Objectives
In the present report, an update of the epidemiological status of canine dirofilariosis in Greece was attempted, in order to draw the possible trends of this parasitic disease in the country. For that purpose, up-to-date results of a currently conducted epidemiological study and previously reported studies on canine dirofilariosis in Greece are presented.

Methods
In the currently conducted survey, blood samples from dogs, originating from areas around the five biggest cities of the country (i.e. Athens, Thessaloniki, Larissa, Patras, Heraklion) were examined. All dogs were older than 1.5 year, living outdoors, not receiving heartworm preventive treatment and included in the survey irrespectively of their clinical condition. The blood samples were examined by the modified Knott’s test and a commercially available serological method (PetCheck® IDEXX). For every dog, data such as sex, breed, age and living conditions were recorded. For the collection of previously reported data on canine dirofilariosis in Greece, electronic academic databases and search engines as well as Books of Abstracts and Proceedings of Congresses were used as source of information.

Results
In the current survey, until now 452 dogs have been examined and the results in the different areas of the country were the following: in Thessaloniki 13 out of 100 (13%), in Patras 3 out of 92 (3.26%) and in Athens 1 out of 160 (0.62%) dogs were positive. In 23 and 77 animals from Larissa and Heraklion respectively, no positive dog was found. Most of the previous surveys on canine dirofilariosis in Greece have been carried out in the northern country where the prevalence is reported between 10.6-34.13%. In a survey conducted in Attica, central Greece, in 2001, only 0.7% of dogs were positive.

Discussion and conclusions
As in other European countries, Greece is divided into two: in the north, canine dirofilariosis continues to be one of the most prevalent parasitic diseases of the dog, while in the south the disease is sporadic. Dirofilariosis prevention, applied with increasing intensity, does not seem to diminish the prevalence, probably due to the presence of untreated stray dogs. The percentage of infection in central and southern Greece remains low, a fact that needs to be studied by extensive entomological and epidemiological investigations. On the other hand, new endemic foci, like the one spotted in Patras, are likely to appear.
HUMAN DIROFILARIOSIS - CLINICAL PRESENTATION AND DIAGNOSIS

Vladimir Kartashev¹,², Tatiana Tverdokhlebova¹, Eduard Yagovkin¹, Nikolay Bastrikov², Denis Dontsov², Yuri Ambalov², Tamara Pavlikovskaya¹, Olga Sagach³, Svetlana Nikolaenko³, Nina Chizh³, Zhambarkh Beksin⁴, Zhanna Shapiyeva⁵, Alla Korzan⁵, Andrey Vedenkov⁵, Boris Ilyasov⁶, Javier González-Miguel⁷, Rodrigo Morchón⁷, Fernando Simon⁷

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⁷Laboratory of Parasitology and IBSAL, University of Salamanca, Salamanca, Spain.

Introduction: Diagnosis of human subcutaneous dirofilariosis (HSD) is accidentally established as a result of a surgery in most cases. Objectives: The aim of the study was to evaluate and to improve the quality of clinical preoperative HSD recognition.

Materials and Methods
We analyzed the clinical data that were available from 2014 patients’ records with HSD whose diagnosis was then confirmed by microscopic examination after surgical removal of the parasite.

Results
The most frequent localization of the parasite was the eyes and periorbital area, found in 36.8% of patients (ranging from 21% to 48% in different years). Diagnosis almost never caused a trouble for a doctor especially in the cases where the parasites were located in the conjunctiva and were available for direct inspection at ophthalmoscopy. The second most affected location was head and neck (27.5%), but the assumption of HSD occurred much less frequently. Trunk location of the parasites was observed in 11.5% of patients. Most of them seek medical care relatively late except in the case of breast area in women (2.7%) when malignant tumor was often suspected as preoperative diagnosis. Subcutaneous location of the parasite in the areas of upper and lower extremities was found in 9.4% and 8.6% of HSD cases, respectively. Localization in genital area was detected in 3% of cases in mostly young men. A high proportion of patients with this localization seek doctor consultation early during the 1-2 week of the illness because in addition to the appearance of the lamp in the scrotum they reported prominent signs of inflammation. In 5 patients, the parasite was removed from the abdominal cavity during surgery for emergency characterized with clinical presentation of peritonitis. Pulmonary nodules probably caused by *Dirofilaria immitis* were detected in another 5 patients. Discussion and conclusions

Analysis of clinical data showed that early medical care seeking was observed in the case of local manifestations of HSD as local inflammatory response. In the case of head and genitals as well as in the mammary gland locations, patients also consulted a doctor early. Late referral to a doctor was observed for trunk and extremities location. Preliminary HSD diagnosis was expected by a doctor in the area where the disease occurs more frequently. Ultrasound when used was the only method of presumptive preoperative HSD diagnosis which allows identifying *Dirofilaria* before surgery.
OVERVIEW OF DIROFILARIOsis IN SERBIA DURING THE LAST TEN YEARS 2004-2014 AND CURRENT STATUS OF THE DISEASE
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5 Institute for Public Health, Medical Faculty, University of Nis, Serbia

Introduction
Dirofilariosis is a vector borne zoonosis mostly caused by *Dirofilaria immitis* and *Dirofilaria repens*. The first dirofilariosis case was reported in Serbia in 1998. The first cases of dirofilariosis were discovered as a side finding during the dissections of dogs. Since 2003/2004, veterinary laboratories started doing a regular routine check in dogs for dirofilariosis.

Objectives
In this paper we would like to give an overview of the situation with dirofilariosis in Serbia as an example of spreading of dirofilariosis to the central parts of Europe.

Materials and Methods
Methods used in all of the laboratory diagnostic were ELISA tests and modified Knott test, which were performed in the blood samples of dogs of different origin and location.

Results
In 2003-2004, seroprevalence for dirofilariosis in dogs was 5.9-7%. In 2006-2007, seroprevalence in dogs with no clinical symptoms was 10-11%. But in dogs with clinical symptoms, seroprevalence was 80%. During 2010, seroprevalence in working dogs went up to 14%, in pet dogs it was 11% but in urban regions it was even 20%. During the period from 2011-2013 five human cases of dirofilariosis were reported in Serbia. Finally, during 2013-2014, 78 samples were analysed for dirofilariosis. From hunting dogs, 59 samples were analysed and 19 samples from working dogs were analysed for dirofilariosis. In total, seroprevalence for dirofilariosis was 26.9%.

Discussion and conclusion
Dirofilariosis in Serbia went from a side finding in dogs, from occasional to frequent finding in dogs with no symptoms, then in dogs with symptoms and finally human cases were found. It has become a significant zoonosis which should be in the focus of veterinarians and medical doctors. During the period of 10 years, seroprevalence in dogs went from 7% to 26.9%. A one health concept and perspective should be performed if we want to win a fight with dirofilariosis. Currently a research among human population is being performed and also research on vectors and presence of *Dirofilaria* in them.

Acknowledgements
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THURSDAY, 3 JULY, 2014 ∙ 17:30-17:45
UPDATING THE PREVALENCE OF CANINE DIROFILARIOSIS IN PET DOGS IN NOVI SAD, VOJVODINA, SERBIA
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Introduction
Nematodes of the genus *Dirofilaria* are currently considered emerging agents of parasitic zoonoses in Europe. Climate changes, the existance of animal reservoirs (domestic and wild canides), and global movement of dogs have caused an increase in the spreading of these mosquito-borne nematodes. Although data concerning prevalence of both *Dirofilaria* infections in Serbia have been published, it is of interest for human and veterinary medicine to follow up these infections among dogs.

Objectives
The aim of this study was to update the prevalence of *Dirofilaria* infections in pet dogs.

Material and methods
From the year 2010 to the year 2014 pet dogs from Novi Sad were tested for *Dirofilaria* infections. This research was done in 128 privately owned pet dogs. At the moment of testing, dogs were at least 7 months old, exposed minimally to one mosquito season and without history of treatment with macrocyclic lactones. All samples were examined by wet blood smears, the modified Knott test and heartworm antigen test (*Canine Heartworm Antigen test* kit, Idexx Laboratories, Inc.).

Results and discussion
Among examined dogs 45 (35.16%) dogs had clinical signs, while the rest of the dogs were asymptomatic. Circulating microfilariae of *D. immitis* were found in 14 dogs, while circulating microfilariae of *D. repens* were found in 22 dogs. Prevalence values for *D. immitis* and *D. repens* were 16.41 % and 17.19 %, respectively. In 8 dogs (6.25%) infection with both *D. immitis* and *D. repens* were detected. Results of this study, compared with results of previous investigations in Novi Sad, shows increase of infection with *D. immitis*, decrease of infection with *D. repens*, and increase of infection with both *D. immitis* and *D. repens*. Further investigations are required with higher number of samples to confirm these findings.

Conclusion
This study reveals a 10.16% prevalence of *D. immitis* as single infection, 10.94% prevalence of *D. repens* as single infection, and 6.25% prevalence of mixed infections with both *Dirofilaria* in pet dogs in Novi Sad.

This work is part of the research done in the project TR31084 granted by the Serbian Ministry of Education and Science.
Canine Filarioidosisis in Lisbon, Portugal, over a 14-year period: prevalence, diagnosis and routine laboratory approaches

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**Introduction:** Canine cardiopulmonary dirofilariosis is a potentially fatal disease, endemic in Portugal. However, other less pathogenic dog filarioid infections may occur in the country, namely Acanthocheilonema reconditum and A. dracunculoides, generating blood-circulating microfilariae. Discrimination between agents is essential for an accurate diagnosis and for choosing the appropriate treatment. Modified Knott’s technique is a rapid, simple and inexpensive diagnostic tool that enables to distinguish between Dirofilaria immitis, D. repens and Acanthocheilonema spp.

**Objectives:** This study aimed to analyse all gathered data regarding canine filarioi infection in the scope of activity of the Laboratory of Parasitic Diseases (FMV-UL), providing a better understanding of its evolution pattern throughout the last 14 years.

**Methods:** Laboratory records were obtained from 2000 to 2013, concerning the Lisbon metropolitan area. Morphometric analysis of blood microfilariae was performed after concentration by the modified Knott’s technique. Body length, diameter and anterior and posterior ends were observed. Statistical analysis was performed with SPSS v.20, using Pearson Chi-square test ($\chi^2$) and considering a significant $P$-value <0.05.

**Results:** A total of 886 dog blood samples were tested, including animals of both gender and ages ranging from 5 months to 14 years old. Of the total samples tested, 14.6% (N=129) were positive for microfilariae, of which 57.4% (N=74) for Acanthocheilonema spp., 36.4% (N=47) for D. immitis and 6.2% (N=8) for Acanthocheilonema spp. and D. immitis co-infection. An average prevalence of 9.3% (82/886) was found for Acanthocheilonema spp. and 6.2% (55/886) for D. immitis. No D. repens microfilariae were found. Due to the overlapping size ranges of A. dracunculoides and A. reconditum, we were not able to distinguish these two species. Detected filarioi co-infections included Leishmania infantum using an indirect immunofluorescence assay and Rickettsia spp. in blood smear.

Regarding seasonality, all years and months registered positive results for Acanthocheilonema spp. and only 2011, 2013 and February were absent for D. immitis. The maximum number of Acanthocheilonema spp. and D. immitis diagnosed infections was found in 2003 (N=17) and 2006 (N=9), respectively. The highest number of Acanthocheilonema spp. and D. immitis infections were recorded in July and March, respectively.

**Discussion and Conclusions:** Considering the enhanced international mobility of companion animals and the emerging potential of Dirofilaria spp., routine filarioi diagnosis is important as a first step test. Our results confirm the endemicity of cardiopulmonary dirofilariosis in Portugal and highlight the need of a systematic screening for this disease together with an effective and targeted preventive therapy.

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THURSDAY, 3 JULY, 2014 ∙ 18:00-18:15
DIROFILARIA REPENS AND DIROFILARIA IMMITIS IN AUSTRIA
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Introduction
*Dirofilaria repens* and *D. immitis* are filarioid helminths with domestic and wild canids as main hosts and mosquitoes as vectors. Both species are known to cause zoonotic diseases, namely pulmonary (*D. immitis*), ocular (*D. repens*) and subcutaneous (*D. repens*) dirofilariosis. Both *D. immitis* and *D. repens* are known as invasive species and their distribution is associated with climate change. Until very recently both species were known not to be endemic in Austria.

Methods
We summarize introduced and possible autochthonous cases of *Dirofilaria* sp. in dogs and humans in Austria. Furthermore epidemiological mosquito studies are discussed.

Results
In Austria most cases of *Dirofilaria* sp. in humans and dogs are introduced. However, rarely infections with *D. repens* were discussed to be autochthonous. The introduction of *D. repens* to Austria was confirmed very recently – Within a mosquito surveillance the parasite was examined in Burgenland (Eastern Austria) for the first time in its vector.

Discussion
The distribution of *Dirofilaria* sp. in Austria remains unclear. However, the first findings of *D. repens* in mosquitoes have shown that the parasite is present in Eastern Austria. However, if the parasite is seen to be endemic has to been discussed.

Conclusion
We summarize the situation of *Dirofilaria* sp. in Austria. The first findings of *D. repens* in mosquito vectors indicate that *D. repens* presumably invaded in Eastern Austria.
FRIDAY, 4 JULY, 2014 ∙ 08:30-09:10 ● Invited Speaker
WOLBACHIA IN CANINE AND FELINE HEARTWORM INFECTION
Laura Helen Kramer
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Introduction
Dirofilaria immitis, the causative agent of canine and feline heartworm disease, harbours intracellular bacteria named Wolbachia pipientis. Indeed, most filarial species studied so far, with very few exceptions, contain these microorganisms which are thought to play an essential role in the biology and reproductive functions of their filarial hosts.

Discussion
Wolbachia pipientis are gram-negative bacteria belonging to the order Rickettsiales. They closely resemble other bacteria belonging to the same group like Ehrlichia spp. and Anaplasma spp. Initial descriptions of bacterial-like structures using electron microscopy and more recent studies by immuno-histology have provided a comprehensive description of the distribution of Wolbachia in Dirofilaria immitis. They are found throughout all the stages of the life cycle of the nematode. In adult D. immitis, Wolbachia is predominantly found throughout the hypodermal cells of the lateral cords. In females, Wolbachia is also present in the ovaries, oocytes and developing embryonic stages within the uteri, whereas they have not been demonstrated in the male reproductive system. The bacterium is vertically transmitted through the cytoplasm of the egg and not through the sperm. All current evidence suggests that Wolbachia is a symbiont in filarial worms: i.e. the presence of the bacteria is essential for the filarial worm’s survival and removal of Wolbachia (antibiotics/radiation) leads to sterility of females and eventual death of adults. Recent studies on the genome of Wolbachia from D. immitis have started to shed light on the interaction between bacteria and filarial worm.

Conclusion
Wolbachia has become an interesting target for understanding the pathogenesis of heartworm disease in dogs and cats and for its treatment and control.
Pathogenesis

The pathological, clinicopathological, and clinical response to infection with *Dirofilaria immitis* in cats is not well understood. The pulmonary arterial response to adult heartworms is more severe than that of the dog, although pulmonary hypertension has infrequently been reported. Dillon demonstrated pulmonary enlargement within one week of transplantation of adults, suggesting an intense host-parasite interaction. A severe myointimal and eosinophilic response produces pulmonary vascular narrowing and tortuosity, thrombosis, and possibly hypertension. Because the feline pulmonary artery tree is smaller than that of the dog and has less collateral circulation, embolization, even with small numbers of worms, produces disastrous results with infarction and even death. Although uncommon, cor pulmonale and right heart failure can be associated with chronic feline HWD and is manifested by pleural effusion (hydro- or chylothorax) and/or ascites. The lung per se also is insulted by HWI, with eosinophilic infiltrates in the lung parenchyma (pneumonitis), pulmonary vasculature, and air spaces. The pulmonary vessels may leak plasma producing pulmonary edema, which has been considered by some to represent acute respiratory distress syndrome (ARDS). If the cat survives this initial insult, type II cells proliferate, replacing damaged type I cells, potentially impairing O\textsubscript{2} diffusion. The end result is diminished pulmonary function, hypoxemia, dyspnea, and cough.

Acute or sudden death is typically associated with worm death and fulminant pulmonary failure, possibly associated with pulmonary embolism. Recent research suggests, however, an immune-mediated reaction to HW antigens in the feline shock organ (lung). Fatal respiratory failure probably results when HW antigen is released, producing bronchiolar and bronchial constriction, pulmonary congestion, superficial pulmonary hemorrhage, and periarterial hemorrhage.

“HARD”. It has been known since 1996 that cats exposed to heartworms but which reject maturation develop radiographic lesions. Recently, studies of both natural and experimental infections have confirmed this finding. In natural infections, pulmonary arterial lesions (myointimal proliferation and thrombotic obliteration) have been demonstrated in HW-free, antibody-positive cats and airway, pulmonary artery and pulmonary interstitial lesions have been demonstrated in cats heavily, experimentally-infected cats in which pharmacological abortion of the infection at the early L\textsubscript{5} stage was performed. This combination of data from experimental and natural infections indicates that when cats abort infections at the early 5\textsuperscript{th} stage, radiographic and histological changes develop in the lungs, likely producing the most commonly recognized signs in cats, cough, dyspnea and wheezing. The American Heartworm Society has utilized this information in a campaign for heartworm awareness, labeling it “HARD” or “Heartworm-Associated Respiratory Disease”. The fact that immature adults that never fully mature can cause disease is important to our understanding of this syndrome. We do not know if the resultant pathology is persistent whether it can result in fatality, and if it explains signs later in the disease course. It also causes confusion as to the exact terminology for HWI as a cat may fall outside the standard concept of “exposed and
uninfected” as opposed to “infected”.

A symbiotic bacterium, *Wolbachia*, is known to occur within filarioid parasites at all stages. This bacterium is essential for filarial reproduction and well-being. It has been hypothesized that antigens from these bacteria are proinflammatory, contributing to the HWD, particularly upon the death of the adult HW and that, possibly, treatment of bacteria with tetracycline might be a strategy in the treatment adult dirofilariosis. Currently, there are no data to support this interesting hypothesis. However, doxycycline coupled with ivermectin has proven beneficial in HW-infected dogs.

**Clinical Signs**

Cats with HWI may be asymptomatic and, when present, clinical manifestations may be either peracute/acute or chronic. Acute or peracute presentation is usually due to worm death, embolization or aberrant migration and signs variably include salivation, tachycardia, shock, dyspnea, hemoptysis, vomiting and diarrhea, syncope, dementia, ataxia, circling, head tilt, blindness, seizures, and death. More commonly, the onset of signs is less acute (chronic form). Reported historical findings in chronic feline HWD include anorexia, weight loss, lethargy, exercise intolerance, signs of right heart failure (pleural effusion; rare), cough, dyspnea, and vomiting. We have found dyspnea and cough to be relatively consistent findings and, when present, should cause suspicion of HWD in endemic areas.

In a report of 50 natural cases of feline HWI seen at North Carolina State University, presenting signs were most commonly related to the respiratory system (32 cats; 64%), with dyspnea (24 cats; 48%) being most often noted, followed by cough (19 cats; 38%), and wheezing. Vomiting was reported in 17 (38%) cats and was noted frequently in 8 (16%). Five (10%) heartworm-infected cats were reported to have exhibited vomiting without concurrent respiratory signs and vomiting was a presenting sign in 7 (14%). Neurological signs (including collapse or syncope, which were described in 5 [10%]) were reported in 7 (14%) cats. Five (10%) of the cats were dead at the time of presentation. Murmurs were infrequently noted in cats that did not have concurrent heart disease, independent of heartworm infection. Heart failure was present in 1 cat but this cat had concurrent hypertrophic cardiomyopathy. Heartworm infection was considered to be an incidental finding in 14 (28%) of the cats in this study. Physical examination is often unrewarding although a murmur, gallop, and/or diminished or adventitial lung sounds may be audible. In addition, cats may be thin and/or dyspneic. If heart failure is present, jugular venous distension, dyspnea, and rarely ascites may be detected.

**Treatment**

The use of arsencial-adulticides is problematic. Thiacetarsemide, if available, poses risks even in normal cats. Turner and colleagues reported death due to pulmonary edema and respiratory failure in 3 of 14 normal cats given of thiacetarsemide (2.2 mg/kg twice over 24 hours). Dillon could not confirm this acute pulmonary reaction in 12 normal cats receiving thiacetarsemide, but 1 cat did die after the final injection. More importantly, a significant, though unquantified, percentage of cats with HWI develop pulmonary thromboembolism after adulticidal therapy. This occurs several days to a week after therapy and is often fatal. In 50 cats with HWI, seen at North Carolina State University, 11 received thiacetarsemide. There was no significant difference in survival between those receiving thiacetarsemide and those receiving symptomatic therapy.
Data on melarsomine in experimental (transplanted) HWI in cats are limited and contradictory. Although there is an abstract report in which 1 injection (2.5 mg/kg; ½ the recommended canine dosage) of melarsomine was used in experimentally-infected cats without treatment related mortality, the worm burdens after treatment were not significantly different than those found in untreated control cats. Diarrhea and heart murmurs were frequently noted in treated cats. A second abstract report, using either the standard canine protocol (2.5 mg/kg twice over 24 hours) or the “split-dosage” (1 injection, followed by 2 injections, 24 hours apart, in 1 month), gave more favorable results. The standard treatment and split-dosage regimens resulted in 79% and 86% reduction in worm burdens, respectively and there were no adverse reactions. Although promising, these unpublished data need to be interpreted with caution as the transplanted worms were young (<8 months-old and more susceptible), the cats may not have had time to develop antibodies to HW antigens, thereby reducing the risk of anaphylaxis, and the control cats experienced a 53% worm mortality (average worm burden was reduced by 53% by the act of transplantation). Additionally, the clinical experience in naturally-infected cats has been generally unfavorable, with an unacceptable mortality. Because of the inherent risk, lack of clear benefit, and the short life expectancy of heartworms in this species, this author does not advocate adulticidal therapy in cats. Surgical removal of heartworms has been successful and is attractive because it minimizes the risk of thromboemboli. The mortality in one case series, using a urethral stone basket, was, unfortunately, unacceptable (2 of 5 cats). We have reported success in cats with heart failure due to HW, retrieving worms with the nitinol snare, using fluoroscopic guidance. This procedure holds promise for the future.

Cats with HWI should be placed on a monthly preventative and short-term corticosteroid therapy (prednisone at 1-2 mg/kg q48h-tid) used to manage respiratory signs. If signs recur, alternate day steroid therapy (at the lowest dosage that controls signs) can be continued indefinitely. For respiratory emergencies, oxygen, corticosteroids (dexamethasone at 1 mg/kg IV or IM or prednisolone sodium succinate at 50-100 mg IV/cat) and bronchodilators (aminophylline at 6.6 mg/kg IM q12h, theophylline sustained release at 10 mg/kg PO or terbutaline at 0.01 mg/kg SC) may be employed. Bronchodilators have logic, based on the ability of agents, such as the xanthines (aminophylline and theophylline), to improve function of fatigued respiratory muscles. In addition, the finding of hyperinflation of lung fields may indicate bronchoconstriction, a condition for which bronchodilators would be indicated. Nevertheless, this author does not routinely utilize bronchodilators in feline HWD.

Recently, doxycycline (10 mg/kg q12h for 30 days) has been used to clear Wolbachia from heartworm-infected dogs to reduce embolic complications, reduce microfilaria numbers more rapidly, and to hasten macrocyclic lactone-induced HW death (“slow-, soft- or trickle-kill”). There are no published studies to indicate that the use of doxycycline in should become routine practice in the management of HWI in cats. In fact, some feel that anything that shortens the life expectancy of the parasite is most likely harmful to the cat. Alternatively, the gradual destruction of the heartworm, associated with loss of the symbiotic bacteria, appears to lessen the response to dying worms, when coupled with ivermectin in dogs with HWI.

The use of aspirin has been questioned as vascular changes associated with HWI consume platelets, increasing their turnover rate and effectually diminishing the antithrombotic effects of the drug. Conventional doses of aspirin did not prevent angiographically-detected vascular lesions. Dosages of aspirin necessary to produce even limited histological benefit approached the toxic range. However, because therapeutic options are limited; because at conventional doses (40 - 80 mg PO q72h), aspirin is generally harmless, inexpensive, and convenient; and
because the quoted studies were based on relatively insensitive estimates of platelet function and pulmonary arterial disease (thereby possibly missing subtle benefits), the author continues to advocate aspirin for asymptomatic cats with HWI. Aspirin is not prescribed with concurrent corticosteroid therapy. Since the vast majority of cats are amicrofilaremic, microfilaricidal therapy is unnecessary in this species. Management of other signs of HWD in cats is largely symptomatic.

**Prognosis**

In the aforementioned study of 50 cats with natural heartworm infection, at least 12 cats died of causes other than heartworm disease. Seven of these and 2 living cats were considered to have survived heartworm disease (lived ≥1000 days). The median survival for all heartworm-infected, cats living beyond the day of diagnosis, was 1460 days (4 years; range 2-4015 days), while the median survival of all cats (n=48 with adequate follow-up) was 540 days (1.5 years; range 0-4015 days). Survival of 11 cats treated with sodium caparsolate (mean 1669 days) was not significantly different from that of the 30 managed without adulticide (mean 1107 days). Likewise, youth (<3 years of age), presence of dyspnea, cough, ELISA-positivity for heartworm antigen, presence of echocardiographically-identifiable worms, or gender of the cat did not appear to affect survival. The effect of HWI on survival has been compared to that of other cardiovascular diseases. Overall, the prognosis for HWI in cats is comparable to that of hypertrophic cardiomyopathy, the most benign of primary feline heart diseases.
RESISTANCE AND HEARTWORM PREVENTIVES: HISTORICAL PERSPECTIVE AND OUTLOOK FOR THE US AND OTHER REGIONS.
Byron L. Blagburn
Auburn University, Auburn, Alabama, USA

Introduction
Reports of heartworm prevention failures with macrocyclic lactone (ML) drugs appeared to be escalating based on a 2005 report by the United States (US) Food and Drug Administration. Most were attributed to client compliance, but many were considered true prevention failures.

Objectives
We assembled a team to investigate selected cases of heartworm prevention failure.

Methods
Approaches and criteria included review of patient records, inability of veterinarians to remove microfilariae using traditionally effective drugs and methods, reduced response to MLs with in vitro microfilaricidals and L₃ bioassays, genetic changes in target genes, and GPS analysis of putative failures.

Results
We have to date identified and maintained *Dirofilaria immitis* isolates in dogs that we consider candidates for resistance to ML preventives. Challenge studies in beagle dogs confirmed resistance in the two isolates tested. *In vitro* bioassay evaluations of susceptibility of these isolates and genetic analysis of target genes yielded results that also were suggestive of ML resistance. Patent infections in experimentally infected dogs on ML therapy were also produced from two different field dogs with suspected lack of efficacy, providing additional support for heartworm resistance. Studies will be presented and discussed in the context of support or lack of support for heartworm resistance.

Discussion
Certain biotypes of *D. immitis* have developed resistance to the ML class of heartworm preventives. However, different formulations of ML preventives can result in different efficacies. These results also support annual antigen and microfilariae testing, as well as additional interventional strategies such as mosquito avoidance and use of ectoparasiticides with claims against mosquitoes.

Conclusions
This research confirms heartworm resistance in some regions of the United States. It is important to point out that documented resistance to heartworm preventives does not confirm or imply resistance to arsenical adulticides. In addition to strategies proposed above, suspected resistant heartworms should be eliminated by appropriate adulticidal and microfilaricidal therapy.
FRIDAY, 4 JULY, 2014 • 11:15-11:30 • Invited Speaker
PREVENTION OF AN EXPERIMENTAL DIROFILARIA REPENS INFECTION WITH A SPOT ON COMBINATION OF MOXIDECTIN 2.5 %/IMIDACLOPRID 10% (ADVOCATE®, ADVANTAGE® MULTI)
Claudio Genchi¹, Marco Genchi¹, Eva Maria Krüdewagen², Gabriele Petry², Roland Schaper²
¹ Department of Veterinary Science and Public Health, University of Milano, Italy
² Bayer Animal Health GmbH, Leverkusen, Germany

Introduction
Dirofilaria repens is prevalent in several regions of the world, including Europe, Africa and Southeast Asia. It is the main agent of cutaneous dirofilariosis in dogs. Humans can be infected as “dead end” hosts with increasing numbers of infections in Europe. As dogs are the main reservoir, one important measure to protect humans from the risk of infection is to control the infection in dogs.

Objectives
To evaluate the efficacy of a spot on combination of moxidectin 2.5% /imidacloprid 10% (Advocate®, Advantage® multi) against experimental D. repens infection in dogs.

Materials and Methods
The laboratory study was carried out in a blinded, negative-controlled randomized design. On study day (SD) 0, 4 male and 4 female 5-6 months old Beagle dogs received a single spot on treatment at a target dose of 2.5 mg moxidectin plus 10 mg imidacloprid kg bodyweight (bw). The efficacy was evaluated by comparison with a placebo-treated negative control group of 8 dogs. On SD 28, each dog was experimentally infected with approximately 75 infective D. repens larvae obtained from infected mosquitoes. General health observations were conducted daily during the entire study period of 246 days. Blood samples for detection of circulation microfilariae (mf) were collected at least every 4 weeks after treatment up to SD 238. A modified Knott test was conducted to detect mf in blood of the dogs. Mf-positive blood samples of control dogs were used for PCR-analysis to further specify the parasite species. On SDs 245 and 246, all dogs were euthanized for necropsy and detection of pre-adult and adult D. repens worms.

Results
Blood samples of all treated dogs were mf-negative at all sampling days up to 238 days (34 weeks) after treatment. Three blood samples out of 8 control dogs were found mf positive on SD 196 and 5 were found positive on SDs 224 and 238. In the mf-positive dogs, individual counts of mfs ranged between 7 to 2800 per ml. The highest mf density was found on SD 238. In 4 out of 5 mf-positive blood samples collected on SDs 196 and 224, D. repens was identified by PCR analysis as the only Dirofilaria species used for experimental infection. D. repens was identified using D. repens–specific primers or a combination of a PCR with pan-filaria-primers and a subsequent DNA sequence analysis. During necropsy, pre-adult and adult D. repens worms were found in each of the 8 untreated control dogs, whereas no pre-adult or adult worm was found in any of the treated dogs. In the untreated control dogs, individual worm counts ranged between 3 to 21 worms per dog. Untreated control group was adequately infected with one dog having only 3 adult worms at necropsy and all other dogs 5 or more worms.
Conclusions

The results of this study showed a high preventive efficiency of a single spot on treatment of moxidectin 2.5%/imidacloprid 10% in dogs experimentally infected with *D. repens*. The Advocate® treated study group was high statistical significant superior vs the untreated group (*p* = 0.0002, MW-statistic = 1.0). The product was well tolerated in all study animals and no treatment related adverse reactions were observed throughout the study.
FRIDAY, 4 JULY, 2014 · 11:30-11:45
NODULAR PRESENTATION OF DIROFILARIA REPENS IN A CAT MIMICKING SOFT TISSUE SARCOMA
1,2IDEXX Laboratories Italia srl, Novara Daily Lab, Novara, Italia
3,4Istituto Veterinario di Novara, Novara, Italia
5 Institut of Parasitology, Veterinary Faculty, University of Leipzig, Leipzig, Germany
6 IDEXXVet Med Laboratory GmbH, Ludwisburg, Germany.

Introduction
Dirofilaria repens is the causative agent of canine and feline subcutaneous dirofilariosis, a mosquito-borne disease that has become increasingly recognized in Europe. D. repens infection is thought not to cause clinical signs in most of animals infected.

Description of the case
A 6 years old, spayed female, domestic Maine Coon cat was presented for multiple subcutaneous nodules. The cat was regularly vaccinated and treated monthly from May to September with selamectin. Physical examination revealed three firm and fixed nodules within the subcutis of the trunk. No clinical symptoms were observed; CBC and serum biochemistry panel showed no abnormalities. Computed tomography (CT) showed three ovular masses infiltrating the muscular plane with axillary and inguinal lymphoadenopathy. A presumptive diagnosis of a soft tissue sarcoma was suggested. Cytological evaluation was consistent with a mixed inflammation with a predominant population of histiocytic cells and eosinophils. One slide showed many microfilariae and multinucleated morulae. Previous diagnosis was discarded and a parasitic origin of the nodules was suspected. At ultrasound examination many double and parallel hyperechoic lines were observed and a mini-invasive procedure to remove nematodes was attempted, but only one adult parasite was successfully extracted. Then, the three nodules were surgically excised. Microfilaremia was not detected on modified Knott’s test, but a slight positivity by means of a pan-filarial PCR was found in a blood sample. Analysis of the sequence revealed 98% identity to Dipetalonema dracunculoides. Antigenic ELISA test for Dirofilaria immitis was negative. Histopathological, morphological and PCR examinations of the adult nematodes extracted from the nodules confirmed that the parasite was D. repens.

Discussion
To the authors' knowledge, this case is the first description of D. repens infection in a cat presenting itself as large and multiple subcutaneous nodules. No microfilariae were detected in blood but sequencing detected the presence of D. (Acantocheilonema) dracunculoides DNA in the blood suggesting a possible coinfection.

Conclusions
D. repens infection in the cat may present itself as multiple and large infiltrating subcutaneous nodules and it should be took in consideration in the differential diagnosis of nodular subcutaneous lesion in feline species.
ANALYSIS OF LABORATORY PARAMETERS IN CASE OF DIROFILARIA REPENS INFECTION
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²Department of Biomathematics and Informatics, Faculty of Veterinary Science, Szent István University, Budapest, Hungary

Introduction
The significance of vector-borne parasitic diseases worldwide increases and in connection with that, also infection caused by Dirofilaria repens emerges more frequently in European dog population. This filarioid nematode lives in the subcutaneous tissue of carnivores, and similarly to Dirofilaria immitis, produces microfilariae that are released into the bloodstream of the host. Data show that 18 % of the dogs living in Hungary are infected with this parasite and in some regions mainly near the rivers the prevalence reaches 20-30 %. The presence of D. repens is mostly revealed only by coincidence, and in most cases veterinarians do not assign much importance to the fact of the infection, because the parasite was formerly thought to be apathogenic. But then subsequent surveys have proven that D. repens should be preferably described as “potentially pathogen”, which gives a justification of further examinations on this nematode. There are barely few publications about the clinical signs of subcutaneous dirofilariosis. Moreover most of these studies describe only individual cases or mention coinfected dogs with other parasites too.

This presentation describes a survey to examine the clinical laboratory findings of dogs infected with D. repens, so as to get a more precise view of the pathological alterations induced by the parasite.

Materials and Methods
Between October 2007 and August 2008, 457 blood samples were examined by modified Knott’s method and 75 of these by PCR technique. Hematological and biochemical results were made in all of the 457 cases. With the aim of collecting more data on the clinical aspects of cutaneous dirofilariosis, we analysed haematological and biochemical parameters of infected and not infected dogs.

Results and Discussion
The prevalence of infection caused by D. repens was determined to be 12.5 % (57 dogs) for the investigated dog population. Our comparative examination of the hematological and serum biochemical profiles of the dogs revealed leukocytosis, eosinophilia, higher liver enzyme values, higher urea value and thrombocytopenia in the infected group compared to those not infected. Only urea level was significantly higher according to statistical analysis, and decrease of thrombocyte number was almost significant.

Conclusion
The alterations of laboratory findings are similar of those in heartworm infected dogs, which may mean that these deviations are caused by microfilariae and not adults.
SEROPREVALENCE OF FELINE HEARTWORM (DIROFILARIA IMMITIS) DISEASE IN CATS OF CENTRAL PORTUGAL

Ana Luísa Vieira1,4,5, Maria João Vieira4,5, João Manuel Oliveira3,4,5, Ana Rita Simões3, Pablo Diez-Baños2, Juan Gestal1


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Introduction

Heartworm infection (Dirofilaria immitis) is now recognized as a potential cause of severe disease in the cat and can be clinical and diagnostic challenge to practicing veterinarians. It is generally believed that in areas where canine heartworm disease is endemic, cats are also at risk of infection, but a much lower rate. Vieira et al (2014) showed a 27.3% of prevalence of Dirofilaria sp. in dogs in the center of Portugal, so it’s expected that cats to be at risk. Objective The aim of the present study was to determine the prevalence and risk factors concerning D. immitis (Leidy, 1856) infection in cats living in Figueira da Foz, located in the central region of Portugal.

Methods

In the period between March 2011 and July 2012, 100 blood samples were obtained from cats over 1 year of age, living in Figueira da Foz, with no previous history of heartworm prevention or diagnosis. Every blood sample was analyzed using several laboratory techniques (direct microscopic evaluation of a fresh blood sample, modified Knott technique, ELISA antigen detection test – Idexx Snap® Feline Triple® Test, and antibody detection test – Heska Solo Step FH®). A thoracic radiography was taken if one of the serological blood exam tested positive. An echocardiography was performed whenever possible in seropositive cats.

Results

A total prevalence of 16% (CI95%: 9.4-24.7%) was found. A total of 16 cats were positive in antibody detection test and two of these cats were positive in antigen detection test. No microfilariae were identified in the direct blood sample and modified Knott technique. Using the Brawner et al (2000), Radiography Heartworm Score Criteria, 37.5% didn’t had radiographic change consistent with Feline Heartworm Disease (FHD) (score 0), 25% had radiographic change consistent but not specific for FHD (score 0,5), 31.25% had radiographic change mildly indicative of FHD (score 1) and 6.25% had radiographic change moderately indicative of FHD (score 2). The echocardiography was performed in 8 cats and the parasite was identified in 4 cats.

This study also pointed the fact that the cats not neutered had higher risk of infection.

Discussion/Conclusion

The results of this study demonstrated, for the first time, the presence of heartworm disease in cats in the center of Portugal. This finding emphasizes the importance of systematic screening for this disease, as well as the need to prevent it in every cats living in this geographic area.

This Work was supported by FCT (Fundation for Science and Technology, Ministry for Science and Technology and Higher Education) scope of a doctoral scholarship SFRH/BD/64692/2009.
FRIDAY, 4 JULY, 2014 ∙ 12:15-12:30
AN ALTERNATIVE METHOD FOR MECHANICAL REMOVAL OF DIROFILARIA IMMITIS IN DOGS USING A HOMEMADE SNARE
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Introduction: Canine dirofilariosis is a life-threatening parasitic disease, increasingly reported worldwide. The main treatment goals are to improve the animal’s clinical condition, eliminating all life stages of heartworms, with minimal post-treatment side effects. This can be achieved through mechanical, surgical or chemotherapeutical approaches. Manual extraction is the preferred method due to its diminished invasiveness, reduced damage of the vascular endothelium and shortened anaesthesia duration. However, it is performed in very few Veterinary centers in Europe and USA and it is more widely applied in Japan.

Description of the case: An elderly non-castrated mixed-breed male dog was presented to the FMV-UL, with a history of severe cough, dyspnoea, syncope and exercise intolerance. The owner reported a recent episode of hind limbs weakness and temporary loss of consciousness. Thoracic auscultation revealed an increased respiratory effort and a loud systolic regurgitant heart murmur on the right side. Thoracic radiography showed a slight dilation of the right ventricle and bulging of the pulmonary arteries. Further transthoracic echocardiography revealed the presence of linear, mobile, parallel hyperechoic structures within the right ventricle and pulmonary artery, consistent with the presence of heartworms. A commercial Dirofilaria kit was performed, supporting the diagnosis of cardiopulmonary dirofilariosis. Heartworm removal surgery was proposed using an effective non-traumatic intravascular snare by adapting common economical coronary wire. A homemade catheter-guided snare was created by folding a 0.014-inch coronary wire (BMW, Abbott Vascular). Percutaneous heartworm extraction was performed by inserting the modified snare into a 6-F Judkins right coronary guiding catheter BMW (Cordis) and advancing it into the right ventricle, under fluoroscopic guidance. Two adult heartworms and several immature stages were successfully extracted without complications. Postoperative treatment was managed with success using melarsomine, ivermectin and doxycycline to assure both adulticide and larvicide treatments and no recurrence after surgical intervention.

Discussion/Conclusion: Our findings suggest that a homemade snare catheter is a safe, inexpensive and effective method for adult heartworms removal, allowing a good rate of success in post chemotherapeutic treatments associated with thromboembolism.

Funding: Portuguese Foundation for Science and Technology through a PhD research grant SFRH/BD/85427/2012. This work was done under the frame of EurNegVec COST Action TD1303.
Introduction
Canine heartworm disease is caused by infection with *Dirofilaria immitis*, a filarioid nematode that resides in the pulmonary arteries and occasionally in the right heart chambers of infected dogs. Specific adulticide therapy for heartworm is melarsomine dihydrochloride, but due to several reasons this therapy can be modified or completely replaced with alternative one, by using doxycycline and ivermectin in combination.

Objectives In this prospective uncontrolled clinical study we evaluated the effect of a combination of doxycycline and ivermectin on microfilaraemia and antigenemia in naturally infected dogs from Novi Sad, Serbia.

Material and methods
Thirteen dogs with natural heartworm infection were included in the study. Therapy protocol consisted of doxycycline (10mg/kg sid for 6 weeks, then alternately 4 weeks without and 2 weeks with medication at the same dosage) and ivermectin (6-14μg/kg biweekly) until 2 consecutive negative results were reported on antigenemia, but not longer then 9 months. The dogs that had been still positive after 9 months were tested again 3 months later. General clinical examination, microfilaraemia (modified Knott test) and antigenemia (Canine Heartworm antigen test kit, Idexx Laboratories, Inc.) were evaluated in dogs monthly.

Results and discussion
Treatment was well-tolerated by all dogs. Clinical signs of illness were registered in 6/13 (46.15%) dogs. In all of these dogs the relief of clinical signs was registered on control examinations. At the time of diagnosing the infection with *D. immitis*, circulating microfilaria were present in 9/13 (69.23%) dogs. One hundred percent of dogs have become negative for circulating microfilariae by 90 day of study, while 2/13 (15.38%) dogs finished the therapy by 180 days and 3/13 (23.08%) dogs finished the therapy by 210 days. By the end of studied period of therapy 7/11 dogs were antigen negative, because two dogs are still under treatment. Similar results are reported with doxycyclin and ivermectinin as medications in cases of both experimental and natural infection with *D. immitis*. Further studies are needed to evaluate the effect of this treatment protocol on morphological and functional status of respiratory and cardiovascular system in patients.

Conclusion
These results indicate that a combination of doxycycline and ivermectin is adulticide in dogs with *D. immitis*, with the therapeutic effect achieved in 63.64% of dogs.

This work is part of the research done in the project TR31084 granted by the Serbian Ministry of Education and Science.
MOLECULAR DETECTION OF CERCOPITIFILARIA SPP. IN IXODID TICKS

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Introduction
Nematodes of the genus Cercopithifilaria (Spirurida, Onchocercidae) are tick-borne transmitted filarioids of mammals. In Europe, three species of this genus have been reported in dogs (i.e., Cercopithifilaria bainae, C. grassii and Cercopithifilaria sp. II) and one in roe deer (i.e., Cercopithifilaria rugosicauda). However, data on tick species acting as natural vector of these parasites are scant, therefore, this study aims to collect more information on the topic by means of a molecular screening for the presence of Cercopithifilaria spp. of tick specimens sampled during different research programs and stored in our laboratory.

Materials and Methods
Genomic DNA was extracted from 560 tick specimens collected in different geographical areas of Italy. Ticks were removed from dogs (n=320) and roe deer (n=240) and were morphologically identified as Rhipicephalus sanguineus s.l. (n=209), Ixodes ricinus (n=208), Dermacentor marginatus (n=59), Rhipicephalus turanicus (n=56), Rhipicephalus sp. (n=8), Pholeixodes hexagonus (n=7), Hyalomma marginatum (n=6), Rhipicephalus bursa (n=6) and Haemaphysalis concinna (n=1). Partial cox1 (~650 bp) and 12S rDNA (~330 bp) gene fragments were amplified using primers and PCR amplification protocols previously described. Amplicons were purified and directly sequenced. Sequences obtained were aligned using ClustalW program and compared among them and with those available in GenBank™ dataset by BLAST analysis.

Results
A total of 25/560 (4.5%) ticks were PCR-positive for Cercopithifilaria spp. to both molecular markers. Among tick species, the most frequently infected was I. ricinus (14/173; 8.1%) collected from roe deer, followed by R. sanguineus (9/209; 4.3%) from dogs, and D. marginatus (2/58; 3.4%) from roe deer. Preliminary sequencing of 15 amplicons revealed the presence of C. bainae and Cercopithifilaria sp. II in R. sanguineus (1/209 and 6/209, respectively); C. grassii was found in D. marginatus (1/58), whereas Cercopithifilaria sp. II (2/173) and C. rugosicauda (5/173) were found in I. ricinus.

Discussion
This study reports original data on the presence of different species of Cercopithifilaria in ticks and provides new insights on affiliation with ticks that could play a role in the transmission of these little known filarioids. Interestingly, C. grassii is herein reported for the first time in D. marginatum from roe deer, although further investigations are needed, mostly considering that this filarioid is regarded as a species affiliated only to dogs.
ANGIOSTRONGYLUS VASORUM – NOVELTIES ABOUT EPIDEMIOLOGY & DIAGNOSTIC TOOLS

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Introduction
Canine angiostrongylosis is a cardiopulmonary disease which can be fatal if left untreated. Due to the high variability of clinical signs and the often chronic and subtle course of the infections, diagnosis of this disease is particularly challenging. Routine diagnosis is currently performed through the isolation of first stage larvae (L1) by larval migration methods (Baermann method) from multiple, fresh faecal samples. However, this method has a restricted sensitivity in case of low worm burdens, and it is unsuitable during prepatency. Novel tools such as serological and biomolecular methods have been described, and they may overcome these shortcomings.

Methods and Results
Experimentally infected dogs were available for a comparative analysis of Baermann results, PCR-based detection of Angiostrongylus vasorum DNA in tracheal swabs, blood and faecal samples, and ELISAs for detecting circulating antigens of A. vasorum or specific antibodies against A. vasorum. Both ELISAs were validated in a field study with sera of dogs from Italy, from which also Baermann results were available, proving their validity as alternative, rapid and affordable tests. A high performance of the serological methods was demonstrated, while test results that relied on the presence of parasite material in the faeces (Baermann examination and faecal PCR) or DNA in sputum or blood showed higher variations. Furthermore, a commercial rapid assay (AngioDetect™ Test, IDEXX Laboratories) for the serological detection of circulating antigen and intended for routine in-clinic diagnosis, showed high sensitivity and specificity, with a delay for detecting experimental infections of approximately 3-4 weeks compared to the ELISA. Thus, ELISAs are valid and affordable tools for individual diagnosis, allowing prompt anthelminthic intervention to prevent the onset of severe pathological changes. They are also very advantageous for mass-screening of dog populations. PCRs may be useful in surveys investigating intermediate hosts, while the kennel-side AngioDetect™ Test represents a very easy and practical tool to be broadly applied in veterinary practices for dogs with suspected angiostrongylosis.

Discussion
A. vasorum is since long known from endemic foci in e.g. south-western France, Ireland and Italy. The parasite has increasingly been reported in extended areas in central and western European countries and also in Canada during the last decades. Very recently, A. vasorum was also detected in the USA. In large scale surveys with canine sera from various European countries, the endemic presence of A. vasorum was confirmed in known regions of Italy, Germany, Great Britain, Hungary, France, Denmark and Switzerland. Seropositivity for both ELISAs varied between 0.3-2.0% in dog populations. Positive animals were distributed over large areas of the investigated countries, and additional cases were detected outside of the known endemic foci as well as in further countries such as Poland and Portugal. In these new endemic areas, it is crucial to disseminate the information of the occurrence of A. vasorum and to increase disease awareness among veterinarians in order to prevent fatal cases. Early diagnosis has a good prognosis for A. vasorum affected dogs through adequate anthelminthic treatment before the onset of pathological changes, which can precede evident clinical signs. The authors kindly thank to Bayer Animal Health GmbH (Roland Schaper), Germany, for the financial contribution and many other persons for their great support and collaboration.
**ANGIO DETECT™ – A NEW TOOL FOR THE RAPID DETECTION OF ANGIOSTRONGYLUS VASORUM INFECTION**

Jiayou Liu, Adam Potter, Ramaswamy Chandrasheka
IDEXX Laboratories, Inc., Westbrook, USA

**Introduction**

Canine angiostrongylosis, caused by *Angiostrongylus vasorum*, is an emerging disease with increasing numbers of diagnosed cases in many parts of Europe, Africa and the Americas. Angiostrongylosis can present a variety of non-specific symptoms ranging from respiratory, coagulopathy, and neurological disorders. Currently, definitive diagnosis of canine angiostrongylosis is by the detection of L1 larvae in faeces. The Baermann technique is frequently used by general practitioners as the method of choice for faecal detection of larvae. However, this method has well documented drawbacks. IDEXX Laboratories, Inc. now offers the Angio Detect Test, an in-clinic test for detection of circulating antigens released by *A. vasorum* adults. The presence of the antigens in canine blood indicates the animal is actively infected with the *A. vasorum* parasite.

**Objectives**

To compare the Angio Detect Test with the conventional Baermann technique for diagnostic sensitivity and specificity and cross-reactivity to other parasites.

**Materials and Methods**

For sensitivity and specificity evaluation, paired faecal and blood samples were obtained from 233 dogs with suspected angiostrongylosis. The faecal samples were analyzed using the Baermann technique by IDEXX Reference Laboratories, UK (ISO 17025:2005 accredited). Sera and/or plasma derived from the blood samples including samples from dogs with confirmed infection by *Crenosoma vulpis* (n=3), *Dirofilaria immitis* (n=79) and *Dirofilaria repens* (n=4), were used for evaluation with the Angio Detect Test. The samples were blinded and randomized before testing.

**Results**

In this population of 233 dogs with suspected angiostrongylosis, the Angio Detect Test was found to have a relative sensitivity of 98.1% and a relative specificity of 98.9% compared to the Baermann technique. The three discrepant samples (two were Baermann negative but Angio Detect positive; one Baermann positive Angio Detect negative) were tested with a plate ELISA test developed and validated by the University of Zurich. In all cases, it confirmed the Angio Detect test results. Samples from dogs infected with *Cvulpis, Dir immitis* and *Dirofilaria repens* were all negative with the Angio Detect Test.

**Conclusion**

The new Angio Detect Test exhibits high sensitivity and specificity compared to the Baermann technique allowing veterinarians to rapidly diagnose canine angiostrongylosis.
ANGIOSTRONGYLUS ‘HOTSPOTS’ IN THE UK – PRELIMINARY EPIDEMIOLOGICAL INVESTIGATIONS
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Introduction
Angiostrongylus vasorum is a parasitic nematode that may cause cardiopulmonary disease, neurological signs and/or coagulopathies in the dog. Our recent nationwide survey established that the parasite was distributed widely in the UK, was being seen in areas where it had not been previously reported and that there were two endemic foci, or ‘hotspots’, of infection in Greater London/SE England and S Wales.

Objective
To assess the extent to which the geographical distribution of hot spots of canine infection with Angiostrongylus vasorum established in the UK on the basis of a postal survey of veterinary practices could be explained by a number of environmental, host and bioclimatic factors.

Materials and Methods
This study was conducted in two stages. Firstly, a number of key environmental, host and bioclimatic factors that might account for hot spots of canine infection were identified. These factors included, though were not necessarily be confined to, host (dog and fox population density and distribution), climatic (temperature and rainfall) and environmental factors (soil pH, slope and type). Secondly, climate forecasting was carried out and interpreted in light of the identified distribution of hot spots of infection.

Results
Water budget values are higher in the West gradually decreasing eastwards. Soil moisture index is low (within “low risk” values) between January and May, increasing in June, July and August with higher values moving in September towards Wales and Devon-Cornwall. Normalized difference vegetation index (NDVI) values are higher in the South West of the UK in particular in spring and summer. The results of the climate forecasting model are summarized visually in a series of maps that provide insight into possible explanations for the previously identified clusters of infection in south-eastern England and South Wales.

Discussion and Conclusion
Our previous postal survey has shown that clinical A. vasorum infection in the UK is not limited to traditional endemic foci. The infection is widespread with clusters of infection in south-eastern England and south Wales. We identify those bioclimatic, host and environmental factors that can more plausibly explain the distribution pattern as reported by practices. Maps with areas more suitable, a-priori, are presented and can be used to alert practitioners in some regions as to the higher risk of infection.

Funding provided by Bayer Animal Health is gratefully acknowledged.
FRIDAY, 4 JULY, 2014 ∙ 15:00-15:15

**OCURRENCE OF ANGIOSTRONGYLUS VASORUM INFECTIONS IN DOGS WITH A RELEVANT CLINICAL PICTURE IN DENMARK**

Jakob Lundgren Willesen, Camilla Rahbek, Jørgen Koch
Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

**Introduction**

*Angiostrongylus vasorum* is a metastrongyloid nematode of dogs and foxes often causing severe respiratory disease and / or haemostatic emergencies in the infected animals. In Denmark, prevalence rates of 2.2-9.7% have been reported in dogs depending on sampling area (non-endemic versus hyperendemic area) and selection criteria (clinical versus non-clinical cases). Updated knowledge of local infection risk is important in the process of clinical decision making.

**Objective**

The aim of the present study was to investigate the occurrence of *A. vasorum* infection in a population of Danish dogs with clinical signs consistent with canine angiostrongylosis (CA) in a known hyperendemic area (Zealand) as well as in a geographical area with sporadic disease occurrence (Funen).

**Materials and Methods**

Between October 2012 and November 2013 a total of 171 dogs were included in the study. Dogs were included if presented with one or more of the following clinical signs relevant for the differential diagnosis of CA; cough, dyspnoea, exercise intolerance, clinical signs of coagulopathy, neurological signs, signs of right-sided heart failure or radiological changes compatible with a pulmonary parasitic infection. Faecal material from three consecutive days was collected and examined using the Baermann method at the Central Laboratory, University of Copenhagen. The study was conducted as a multicentre study including 16 veterinary clinics and hospital.

**Results**

Of the 171 dogs included in the study, *A. vasorum* was diagnosed in 24 dogs (14.0%). Further findings were *Crenosoma vulpis* 1.8% (n=3), *Uncinaria stenocephala* 3.5% (n=6), *Toxocara canis* 3.5% (n=6), *Strongyloides stercoralis* 0.6% (n=1). Soil nematodes were found in 7.6% (n=13) of the cases. Occurrence of *A. vasorum* depended on geographical location with highest occurrence recorded in Northern Zealand 27.3% (9/33), Copenhagen area 12.1% (11/91), Funen 10% (1/10) and Southwest Zealand 8.1% (3/37).

**Conclusion**

Fourteen percent of the included dogs were diagnosed with *A. vasorum* in this study which is higher than previously reported although with a marked geographical variation. Even though these clinical signs are not pathognomonic the results shows that this clinical manifestation should rank CA at the top of the differential diagnosis list also in areas outside known hyperendemic foci.

The study was funded by a Master Thesis Research grant from Department of Veterinary Clinical and Animal Sciences, University of Copenhagen and was furthermore supported by a research grant from Bayer AnimalHealth.
FRIDAY, 4 JULY, 2014 · 15:15-15:30
LUNGWORM (ANGIOSTRONGYLUS VASORUM, CRENOBOMA VULPIS, EUCOLEUS AEROPHILA) INFECTIONS IN RED FOX POPULATIONS IN SOUTH WEST GERMANY
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Introduction
The spread of red foxes (Vulpes vulpes) from sylvatic to urban areas is a well-known phenomenon to occur in Central Europe. With their urbanization, foxes import infectious parasitic stages in close proximity to humans and pets. Especially life-threatening parasites, such as Echinococcus multilocularis for humans or Angiostrongylus vasorum for dogs, may be of major concern. So far, data on the latter parasite in foxes in Germany are scarce.

Objective
Therefore we conducted a study on fox parasites covering the period of one year.

Materials and Methods
Overall, pluck (heart and lung) and rectum contents of 569 foxes were collected in three Federal States of Germany (Hesse, Thuringia, Rhineland-Palatinate). Lungs, hearts and adjacent vessels were processed for adult nematode detection. Parasitological diagnoses of faecal samples were performed by SAF technique and Giardia/Cryptosporidium-coprotantigen-ELISAs. Since all samples had been frozen at -80°C as precautionary measure, no Baermann funnel assays could be performed for lungworm larvae detection.

Results and Discussion
Foxes proved heavily infected with lungworms: 69.2 % of the foxes carried adult Eucoleus aerophila, in 32.3% of the samples C. vulpis were found. Whilst Filaroides spp. were rarely diagnosed (0.4%), A. vasorum was detected in 14.1% of the samples indicating that red foxes have to be considered as eminent reservoir hosts for A. vasorum in Germany. Significant regional differences concerning A. vasorum infections were evident with highest prevalences in Rhineland-Palatinate (27.3%), followed by Hesse (19.1%) and Thuringia (8.4%). A. vasorum and C. vulpis were detected throughout the year with C. vulpis infections tending to be more frequently during spring and late summer.

In addition, an A. vasorum- and C. vulpis-specific duplex-Realtime-coprop-PCR was developed. Therefore, a C. vulpis 18S- and 28S-rRNA gene based PCR system was used and combined with a recently published A. vasorum-specific assay (Jefferies et al., 2011). PCR analyses of the faecal samples revealed a total of 65 (11.5%) A. vasorum-positive and 94 (16.7%) C. vulpis-positive samples indicating the general usefulness of this molecular technique in the case of “non-fresh” samples, but also confirming pulmonary sections as superior method for diagnostics.
FRIDAY, 4 JULY, 2014 ∙ 16:00-16:15
UK: NORTHWARD SPREAD OF ANGIOSTRONGYLUS VASORUM – DATA FROM DOGS AND FOXES
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Introduction
Originally restricted to ‘disease hot spots’ in Cornwall (Truro area) and to south Wales (Swansea), Angiostrongylus vasorum became widespread and common in the south-east of the UK since 1990. There has been increasing evidence of spread northward in recent years with cases in dogs reported around cities such as Liverpool and Manchester. Infections in dogs mainly occur where infected Red foxes are have been recorded; assessing the geographical distribution of, and degree of infection in the fox populations may be used to indicate increases in parasite range and to predict potential future foci of infection. This talk presents data on A. vasorum and other lungworms in northern foxes and dogs.

Objectives
To use coproanalysis to estimate prevalence of patent lungworm infections in owned dogs in Liverpool and in rescue dogs from Manchester and Liverpool and to compare results to data from Swansea. Similarly, to process faecal samples and the hearts and lungs of northern foxes to indicate any changes in parasite range since a previous comprehensive surveillance study published 6 years ago.

Materials and Methods
A total of 800 random faecal samples were collected in February, April, May, July and August 2013 and analysed using a concentration device incorporating a sieve of 400µm diameter suitable for recovery of parasite stages from stored samples. 116 samples from Rescue centres were also examined during the same period. 128 faecal samples from northern foxes (culled between 2012-2014) donated by FERA (DEFRA, UK) were similarly processed and 11 entire foxes subjected to post mortem.

Results
Mean % PI with A. vasorum was 0.75 in the random dog population in Swansea. A. vasorum was not detected in dogs from Liverpool but eggs of E. aerophilus were found in one faecal sample. Lungworm infections were not detected from any rescue centres, although the latter location accounted for a statistically significant increase in observed roundworm (PI, 4.5%; P= 0.021) and hookworm infection (PI, 1.8%; P=0.04). Overall, A. vasorum larvae and E. aerophilus eggs were detected in 1.6% and 44% of fox faecal samples respectively. A. vasorum adult worms were recovered from 3 of 11 foxes at post mortem, all originating from the same grid reference.

Discussion and conclusions
A. vasorum was not detected in dog populations in northern cities but future surveillance using fresh faeces or recent antigen detection methods is required. The results here demonstrate that northern areas of the UK can support parasite transmission in foxes and are indicative of range expansion.

Acknowledgments: This work was kindly supported in part by Bayer AH
Diagnosis of French Heartworm and Lungworms in Swedish Dogs: Results from 2011-2013

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Introduction

Angiostrongylus vasorum in Sweden has been detected for the first time in foxes in 2003, in the island of Sydkoster in the county of Bohuslän. In September 2009 the parasite was detected for the first time on Swedish mainland, in a fox found dead in the municipality of Osby (county of Skåne). In the autumn 2011 A. vasorum was detected in a fox in the municipality of Växjö (county of Småland). In Denmark the parasite is stably present in local foci of infection and it represents a large problem in dogs in some areas.

Lungworms (Crenosoma vulpis) in Sweden have always been commonly reported in foxes and regularly in dogs, even if details of the prevalence of infection are not known.

Objectives

The objective of the present abstract is to report the occurrence of both A. vasorum and lungworms in Swedish dogs, showing data obtained from the diagnostic section of Parasitology of the National Veterinary Institute.

Materials and Methods

Baermann test is performed on three fecal samples collected from three consequent days, since larval shedding may be irregular. Samples are pooled and suspended in lukewarm water in a funnel and 24 hours later the sediment from the bottom of Baermann apparatus is examined.

1157 canine fecal samples mainly submitted with the suspect of lungworm infection were analyzed in the period 2011-2013, with an increasing trend of samples examined over the years: 298 (2011), 355 (2012), 504 (2013).

Results

One sample in 2011 and one in 2012 were positive for A. vasorum, while in 2013 four samples resulted to be positive. The percentage of positive samples is not exceeding 0.8%, and all except one are still concentrated in Southern Sweden.

The more common canine lungworm, C. vulpis, was found respectively in 28 (2011: 9.4%), 31 (2012: 8.7%) and 49 (2013: 9.7%) samples, confirming the circulation of the parasite between foxes and dogs at an average prevalence of 9.2%.

Discussion and conclusion

Present data demonstrate that that while lungworms continue to be reported with a stable prevalence/incidence, A. vasorum has increased its spreading, especially reaching northern locations, like Sundsvall. The increased attention towards A. vasorum since its first description in Sweden first led to an increase of analyzed samples and therefore to an increase in the number of findings. Some more data from foxes, as well as serological investigations on dogs should be carried out in order to gather more complete data on the prevalence of A. vasorum.
RESPIRATORY NEMATODES IN DOGS FROM CENTRAL ITALY

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Introduction
Despite Capillaria aerophila (syn. Eucoleus aerophilus), Capillaria boehmi (syn. Eucoleus boehmi), Angiostrongylus vasorum and Crenosoma vulpis are increasingly reported parasites across dog populations of several countries, including Italy, knowledge on their actual distribution is poor. Aim A copromicroscopic survey was conducted in dogs living in central Italy with the aim to provide new insights into the epidemiology of these extra-intestinal parasites in Italy and to improve knowledge on these infections among vet practitioners.

Materials and methods
Five hundred thirty individual faecal samples were collected between November 2012 and December 2013 and copromicroscopically processed with a flotation using a zinc sulphate solution and the Baermann's technique. The stool samples belonged to 450 asymptomatic dogs living mostly in shelters or breedings, and to 80 privately owned animals brought to visit at teaching hospitals or private practices for different respiratory signs. Individual anamnestic data were collected through an interview with the owners and used for the risk analysis performed by binary logistic multiple-regression models.

Results
Overall 75 out of the 530 examined dogs (14.15%) showed to be positive for eggs and/or larvae of respiratory nematodes. In particular, 18 dogs (3.4%) scored positive for larvae of A. vasorum. Five dogs with angiostrongylosis showed respiratory signs (e.g. cough, dyspnea and breathing sounds) and two of them also coagulopathies (e.g. bleeding diathesis). Larvae of C. vulpis were detected in 8 dogs (0.75%) even though respiratory distress occurred only in one dog. Eggs of C. aerophila and C. boehmi were detected in 40 dogs (7.54%) and 31 (5.84%) stool samples, respectively. Twelve and 18 dogs infected with C. aerophila and C. boehmi showed respiratory symptoms, being the most common dry cough, sneezing and hyposmia/anosmia. Mixed infections were found in 22 out of the 75 positive dogs (29.33%). The most common parasitic association was between C. aerophila and C. boehmi, which occurred in 20 animals, 10 of which were symptomatic. The rates of prevalence of C. boehmi and C. aerophilawere significantly higher than those found for A. vasorum and C. vulpis. Also, A. vasorum showed to be more prevalent than C. vulpis even though this result was not statistically significant. The statistical analysis showed that the prevalence rates of C. aerophila and C. boehmi infection were influenced by the type of housing and attitude of dogs and that the presence of respiratory signs were related to the occurrence of these nematodes. A higher prevalence of these capillarids was significantly found in kenneled dog, in hunting and symptomatic animals. Presence of symptoms and hunting activities were risk factors also for A. vasorum infection.

Discussion and conclusion
The present results confirm that extra-intestinal parasites of the respiratory tract are frequent in canine populations of central Italy and underline their clinical importance.
AELUROSTRONGYLUS ABSTRUSUS: NOT JUST PNEUMONIA; TWO CLINICAL SYNDROMES ASSOCIATED WITH AELUROSTRONGYLUS ABSTRUSUS INFECTION
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Introduction
Aelurostrongylus abstrusus is a nematode parasite of the feline respiratory tract with a worldwide distribution. Infection may be emerging and underdiagnosed. The clinical features of infection remain ill-defined with description limited to experimental infections, single case reports or small case series. Reported symptoms mostly are respiratory in origin, namely cough, dyspnoea and tachypnoea. Of late our group has come across two unusual clinical syndromes associated with natural A. abstrusus infection; pulmonary arterial hypertension (PAH) and dyspnoea in the perioperative period (DitPP).

Objective
To describe the clinical features of A. abstrusus associated PAH and DitPP.

Materials and Methods
The medical records of twenty nine cats diagnosed with A. abstrusus in Dierenkliniek Rijen between October 2009 and April 2014 were reviewed. Clinical, diagnostic, diagnostic imaging, treatment and follow-up data were reviewed where available.

Results
PAH was diagnosed in three animals (age 3-6 months). All were dyspnoeic. One presented with abdominal distension and ascites due to congestive right sided heart failure. Thoracic radiography showed predominantly diffuse interstitial and peribronchial changes. Echocardiography showed right heart enlargement, pulmonic artery dilation and moderate to severe pulmonary hypertension. One animal died. In both surviving animals clinical, radiographic and echocardiographic changes were completely reversible after anthelminthic treatment. Acute onset of DitPP was found in eight animals (7-125 months). Thoracic radiographs were available in 6/8 animals. A mixed interstitial, alveolar and peribronchial pattern was visible, as were (acute) alveolar changes in the caudodorsal lung fields. All animals were difficult to ventilate and became hypoxic during anaesthesia. Prolonged recovery with supplemental oxygen lead to resolution of both dyspnoea and the alveolar infiltrates.

Discussion
Diagnosis of pulmonary hypertension is rare in cats. It has only been reported in association with congenital heart disease, pulmonary embolism, chronic upper airway obstruction and heartworm disease. In a recent report on anaesthesia related mortality, A. abstrusus was found in lung tissue 10% of animals. We also found serious anaesthesia related problems and acute onset of non-cardiogenic pulmonary oedema. Both PAH and DitPP associated with A. abstrusus have not been reported before. How A. abstrusus leads to PAH and/or DitPP remains to be elucidated.

Conclusions and clinical relevance
In cats with pulmonary hypertension, right sided congestive heart failure, dyspnoea in the perioperative period and non-cardiogenic pulmonary oedema, A. abstrusus should be considered to be a differential diagnoses. With prompt diagnosis and appropriate treatment, severe clinical and diagnostic imaging changes, might be completely reversible.
Introduction
Recent climate change and the related modification of ecosystems, together with continued strong anthropogenic pressure, have caused the spread of many zoonotic parasitic diseases beyond their long known endemic foci. Such a trend has been documented during the last decade also in metastrongyloid nematode *Angiostrongylus vasorum*, which causes severe cardiopulmonary disease in dogs. While in the past was its occurrence limited to isolated foci in France, Denmark and United Kingdom, in recent years has the parasite emerged in several countries of Western and Central Europe (Italy, Germany, the Netherlands, Poland, Hungary etc.).

Description of the cases
In 2013, the parasite was recorded for the first time in Slovakia. The first case was a 7-month dog without clinical signs, in which the larvae of *A. vasorum* were found by routine examination of faeces. The second case involved an 18-month Bernese mountain dog and was characterized by severe clinical signs with nearly fatal course. The dog suffered from loss of appetite, in sequence the weight loss of 14 kg appeared, associated with strenuous walking and hunched posture. Two times even the acute physical collapse occurred. Hemorrhagic diarrhoea, intense salivation, vomiting, difficulties with urination and haematuria were also reported. Scleral bleeding was evident bilaterally. The dog suffered from severe irritating cough and dyspnoea. Infected dog excreted *A. vasorum* L1-larvae in high numbers – in 10 g of the faecal material more than 800 larvae were counted.

Discussion
Both infected individuals were stray dogs in the past and adopted by new owners. Of importance is the fact that both of them came from the Kosice region in the south-eastern part of Slovakia is characterized by specific ecological conditions suitable for the survival and reproduction of many parasite vectors - from mosquitoes, ticks over to snails. The second dog with severe clinical manifestations and high intensity of infection was trapped directly in the Eastern Slovak Lowland, known as the endemic area of several infectious diseases, including canine babesiosis and dirofilariosis. In terms of circulation of the vector-borne parasites, the significance of the locality is evidenced by the fact that till the 60’s of 20. century this was considered an endemic area for malaria.

Conclusion
The first findings of *A. vasorum* in Slovakia prompted the beginning of the initial epidemiological survey that will be extended to other lungworms of domestic and wild carnivores.

This study was supported by Science Grant Agency VEGA No. 2/0011/12.
P1
CANINE FILARIOID INFECTIONS IN AN AREA OF NORTH-WEST ITALY (LIGURIA) TRADITIONALLY CONSIDERED FREE FROM THE DISEASE
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3Istituto Zooprofilattico Sperimentale of Piedmont, Liguria, Aosta Valley, via Nizza 4, 18100 Imperia, Italy.

Introduction Canine filarioid infections are widespread throughout the world and steadily increasing despite prophylaxis. New cases in dogs and in humans have occurred in many countries and regions that were previously considered to be free or for which epidemiological data were not available. The aim of this work is to report the presence of different species of filarioid nematodes in dogs in an area of north-west Italy (Liguria), traditionally considered free from the disease and where prophylaxis is not implemented.

Methods Between 2009 and 2012 blood samples were collected from 365 dogs living in rural areas of Liguria (in the provinces of Savona and Imperia) and submitted to Knott’s test, histochemical staining, PCR targeting part of the 5.8S-ITS2-28S gene, and ELISA for Dirofilaria immitis antigens (Dirocheck, Synbiotics®). In addition serological data were analysed for D. immitis antigens that had been collected by the Istituto Zooprofilattico Sperimentale (IZS, section of Imperia) between 2004 and 2013 during the annual control for leishmaniosis. An average of 1285 dogs/year (range 417-2089) from the four Ligurian provinces (Imperia, Savona, Genoa, La Spezia) had been tested.

Results Thirty-five dogs were positive to Knott's test for microfilariae (prevalence 9.6%, 95% Confidence Interval: 6.6-12.6%): Acanthocheilonema reconditum was the most common species (8.0%), while Dirofilaria repens (1.4%) and D. immitis (0.6%) were less common. Co-infection by D. repens and A. reconditum was observed in one dog. All morphological identifications were confirmed by histochemical staining and PCR followed by sequencing. Five dogs out of 365 (prevalence 1.4%, 95% CI: 0.2-2.6%) were positive to the ELISA test for antigens of D. immitis. Of these, two dogs were positive to Knott's test for microfilariae of D. immitis, while the other three did not present circulating microfilariae (occult filarioidosis). Analysis of the IZS data showed an average annual serological prevalence of 0.6% for D. immitis in the whole region.

Discussion and conclusion The results of this study confirm that canine filarioid infections are increasing in Italy in areas that were once free, as is happening in many other countries worldwide, in particular in Central and Eastern Europe and South America. Prior to the present study, in our study area there had been only a few reports of cases of unidentified microfilaraemia in 1986 and of one case of D. repens in a dog in Genoa in 1995. This study contributes to the epidemiological map of filarioid infections in Italy, and suggests the need for prophylaxis in this region. In addition, the widespread presence of different species of filarioid nematodes in the provinces of Imperia and Savona suggests the need to enlarge the study using Knott’s test and PCR also in dogs living in the other districts for which only preliminary serological data concerning D. immitis are available. The zoonotic potential of these parasites must also be considered in relation to tourism in the area, which attracts many visitors from other European countries, often travelling with dogs.
P2
CASES OF DIROFILARIOSIS IN DOGS AND CATS IN BUCHAREST AND SURROUNDING AREAS
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Introduction
Dirofilariosis can be responsible for sudden death in dogs, that can also involve cats, and has a zoonotic potential. The present study is a retrospective analysis of the cases diagnosed with dirofilariosis between May 2004 and April 2014.

Materials and Methods
Dirofilaria immitis and Dirofilaria repens were the cause for a total of 96 cases (94 dogs and 2 cats) diagnosed with dirofilariosis and microfilaremia. The methods used in the present study consisted of necropsic and cytological examination (blood and lesions smears). The investigation criteria were related to breed, gender, age, and seasonal occurrence of the disease of the studied animals. In addition, aspects of gross lesions or incidental findings of heartworm and subcutaneous dirofilariosis were researched.

Results
During the studied period, the cases of dirofilariosis summed 2% of all dogs diagnosed in the Pathological Anatomy Laboratory of the Faculty of Veterinary Medicine, Bucharest. Of the total 96 cases, D. immitis was found in 62 dogs (65%), D. repens in 5 dogs (5%), and microfilariae in 27 dogs (28%) and 2 cats (2%). The most affected breeds were the common breed (44%), the German Shepherd (27%) and Mioritic Shepherd (7%), and the males accounted 65% of cases. The mean age was 7.7 years, with a range between 1 and 15 years. The most cases (67%) ranged between 5 and 10 years of age. The season with most diagnosed cases (40%) was spring. During the last 2 years, the disease was found in 59% of all studied cases. The gross findings was dominated by cardiopulmonary lesions such as right heart dilation with left heart hypertrophy, severe pulmonary oedema, thromboembolic parasitism in the pulmonary arteries and red pulmonary infarcts. Occasionally, endocarditis, spleen and kidney congestion and infarction and serosanguinolent effusions were revealed. Subcutaneous lumps and adult parasites were depicted in D. repens parasitism. Cytology was applied to cavitary effusions, blood and neoplastic or non-neoplastic samples. Incidental findings using fine needle aspiration in cats revealed microfilariae in skin lesions. Typical findings of heartworm were displayed in 60% of necropsy cases, while 40% were incidental.

Conclusion
In Bucharest and surrounding areas, the cases of dog dirofilariosis accounted 2% of the last 10 years case history diagnosed in the Pathological Anatomy Laboratory. Heartworm disease showed classical lesions in 60% of the necropsy cases, whereas 40% died from different causes. Microfilariae were highlighted in cytology from both peripheral blood and neoplastic or non-neoplastic lesions in dogs and cats.
PREVALENCE OF DIROFILARIA IMMITIS IN STRAY DOGS IN ERZURUM PROVINCE, TURKEY

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Introduction

Dirofilaria immitis is an important parasite in carnivores and has a zoonotic potential. The aim of this study was to carry out a molecular survey for the presence of D. immitis infection in stray dogs using PCR assay.

Materials and Methods

A total of 133 whole blood samples were collected from stray dogs (105 females and 28 males) in Erzurum, Turkey between April 2013 and April 2014. Of the sampled dogs, 41 (30.8 %) were 0.5-1 years old, 65 (48.9 %) were 1-3 years old and 27 (20.3 %) were 3-8 years old. For molecular identification, species-specific primers which amplify a 203 base-pair fragment of the cytochrome oxidase subunit 1 (COI) gene, were used.

Results

Two (1.5 %) of the 133 samples showed a positive reaction by PCR. No statistically significant differences were observed in relation to sex (P>0.05). The prevalence of D. immitis in dogs aged 3-8 years old was significantly higher (P < 0.05) than other age groups.

In conclusion, D. immitis infection in stray dogs was present in Erzurum province but the prevalence (1.5 %) was too low. It must be related with the mosquito intermediate host which has a temperature dependent development and Erzurum province has relatively short summer times.

The study protocol was approved by the Animal Care and Use Committee at Ataturk University (2012/50 decision number).
P4
PREVALENCE OF DIROFILARIA IMMITIS IN PET AND STRAY DOGS IN BELGRADE AREA IN PERIOD 2012-2013
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6Veterinary Ambulance Artvet, Belgrade, Serbia

Introduction
Dirofilarioses are mosquito-borne parasitic diseases of dogs caused by Dirofilaria immitis and D. repens. D. immitis inhabits the right ventricle, pulmonary arteries, rarely cavum thoracis, bronchus and other organs. On the contrary, D. repens resides in the cutaneous/subcutaneous tissue of the animals. Dogs are usual host of both species but they be occurred in other mammals like cats, foxes, jackals and etc. From 1998 until today in the territory of city Belgrade the high prevalence of dirofilariosis in dogs are established.

Objective
The aim of this study is to present the prevalence of dirofilarioses in pets and stray dogs from capital city of Serbia in period 2012-2013.

Methods
In period 2012-2013, 287 blood samples of pet dogs with clinical signs of heart failure and 110 blood samples of symptomatic and asymptomatic stray dogs from 3 dogs' shelters from Belgrade area were examined. The present symptoms in testing dogs was moderate to severe including chronic cough followed by dyspnoea, swelling of the abdomen, anorexia, weight loss, haemoptysis. Knott technique and Giemsa stained method were applied for determination of microfilariae in blood smears. For detection of antigen of D. immitis adult female in peripheral blood of dogs immunodiagnostic kit IDEXX 4Dx tests was used.

Results
Two species, D. repens and D. immitis were present, also in co-infection in both groups of dogs. The prevalence of D. immitis and mixed infection caused by D. immitis/D. repens was proved in 24.04% (69/287), and 4.18% (12/287) of pet dogs respectively. As well the very high prevalence of D. immitis infection was detected in stray dogs [D. immitis - 29.09% (32/110), mixed infection -9.09% (10/110)].

Conclusion
In the Belgrade district the D. immitis infection rates ranging from 22.01% to 25.3% in period of last 17 years (Pavlović et. al.2007, 2009 2012). Comparing of obtained results with previously findings showed that the prevalence remained at approximately same level as it was in last period.
P5
SEROLOGICAL SURVEY ON CANINE HEARTWORM (DIROFILARIA IMMITIS) INFECTION AND OTHER VECTOR-BORNE PATHOGENS IN DOGS FROM BUCHAREST, ROMANIA
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Introduction
Heartworm infection is a potentially fatal disease in dogs and cats caused by Dirofilaria immitis. The adult worms are located in the pulmonary arteries and the right side of the heart and cause a severe and life-threatening condition known as canine and feline heartworm disease. Dirofilariae are endemic in the Mediterranean countries, particularly in Italy, Spain, or Greece. They are considered as emerging pathogens currently increasing their geographical range. In Romania, the epidemiologic status of dirofilariosis is currently undergoing rapid evolution, as reported in other European countries. The aim of the present study was to determine the prevalence of heartworm infection in dogs in southeastern Romania.

Materials and Methods
A total of 75 dogs from Bucharest’s adjacent area were included in the study. All dogs were older than 1 year, without previous chemoprophylaxis or treatment by microfilaricide products. The age of dogs varied from 12 months to 15 years. Of them, 67 were stray dogs from a shelter and the remainders were mixed-breed companion dogs brought to the clinic for various surgical procedures. The EDTA-blood samples collected from dogs were screened for simultaneous qualitative detection of circulating D. immitis antigen, and antibodies to Ehrlichia canis and E. ewingii, Borrelia burgdorferi, and Anaplasma phagocytophilum and A. platys, using SNAP®4Dx®Plus test (IDEXX Laboratories). Additionally, all dogs were randomly screened for microfilariae by means of a modified Knott test.

Results
Overall, 21 dogs (28.0% [CI=18.24-39.57]) were serological positive D. immitis, being the most prevalent pathogens detected, followed by Anaplasma spp. which was detected in four dogs (5.3% [CI=1.85-13.13]). There were no evidences for Borrelia and Ehrlichia infections. As well, co-infections were not found in this study. D. immitis microfilariae were detected in 14 out of the 21 Ag positive samples. In one samples co infection D. immitis + Acanthocheilonema spp. was detected. The modified Knott test revealed also four samples (5.3%) positive for D. repens microfilariae, of which two were co infections D. repens + Acanthocheilonema spp., and three samples (4.0%) positive for Acanthocheilonema spp. Not a single dog showed any clinical symptoms incidental with the infection.

In conclusion, the findings of the present study emphasize increasing risk for emerging canine heartworm infection in southeastern Romania. Moreover, the findings outline the need for further in-depth studies to assess the risks for the both animal and public health.
P6
MAPPING AND MODELING DIROFILARIA INFECTIONS IN EUROPE
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Introduction
Climate change and increasing temperatures are a global phenomenon that can influence the dynamics of a number of hematophagous arthropods, vectors of pathogens with importance in human and veterinary medicine. In fact, climatic changes, together with an increase in the movement of dogs across Europe, have caused an increase in the geographical range of Dirofilaria immitis and D. repens infections.

Objective
The aim of this study was to verify if the climate of the last 30-years (1980-1989, 1990-1999 and 2000-2012) was such as to facilitate the spread of parasites and allow an increase in the period transmission during the season at risk.

Methods
Geographic Information System based on thermal regimen was constructed to identify areas potentially suitable for Dirofilaria transmission in Europe. These models are based on evidence that: i) there is a threshold of 14 °C below which Dirofilaria development will not proceed in mosquitoes; ii) there is a requirement of 130 growing degree-days for larvae to reach infectivity, and; iii) there is a maximum life expectancy of 30 days for a mosquito vector. The output of these models predicted that the summer temperatures (with peaks in August) are sufficient to facilitate extrinsic incubation of Dirofilaria even at high latitudes. Recently, an additional model was constructed to verify the influence of temperature in the course of three decades (1980-1989, 1990-1999 and 2000-2012) on the risk of infection by Dirofilaria in Italy.

Results
The results showed an expected increasing trend of temperatures, an increase of the Dirofilaria generation numbers into the mosquitoes and a significant extension of the infection risk from 5-6 months (1980-1989) to 6.5 months (1990-1999), up to more than 7 months (2000-20012).

Conclusion
These findings show that geospatial tools are very useful for mapping, monitoring, forecasting and surveillance of both heartworm and subcutaneous dirofilariosis.
HUMAN DIROFILARIA REPENS INFECTION IN UKRAINE (1997–2013)
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Introduction
The filarioid nematode *Dirofilaria repens* is an etiological agent of dirofilariasis, a vector-borne zoonosis. The number of infections in Europe has been increasing recently and *D. repens* has become one of the most rapidly expanding parasites of human and animals.

Objectives
In Ukraine, dirofilariasis has been known for a long time. The first case of human *D. repens* infection was reported in 1927, 16 cases were described in the literature through 1974. From 1975 the cases of human dirofilariasis are a subject of epidemiological registration in Ukraine and are recorded in the state register that covers all country (Salamatin et al. 2013; Acta Parasitologica 58(4): 592–598).

Methods
The epidemiological data covering the period of 1997–2013 have been analyzed. Data have been collected from the reports of all 27 regional sanitary-epidemiological stations owned by the Ministry of Health of Ukraine. A detailed analysis of clinical data gathered during the last 5 years (2009–2013) was conducted.

Results
Within the period of 17 years, 1997–2013, 1717 confirmed human cases of *D. repens* dirofilariasis have been registered. Infections were noted in all of the oblasts of Ukraine, plus Crimea, Kyiv, and Sevastopol. The majority of the cases were noted in Kyiv (176), then the oblasts of Donetsk (158), Zaporizhzhya (149), Dnipropetrovsk (147), Kherson (121), Mykolayiv (113) and Chernihiv (111). Analyzing clinical data of 1007 cases (years 2009–2013), it was found that in 662 cases (66%) the parasitic lesions were located in the head, including 414 (41%) cases of lesions around the eyes. Dirofilariasis of the limbs and torso constituted a lower percentage of cases – 15% and 11% respectively. *D. repens* were also detected in the sexual organs of men (4%), and in female mammary glands (3%). In 17 cases (2%) the location of the parasite was not specified in the data. The age of patients was from 11 months up to 90 years.

Conclusions
The results of our analysis point to a steady increase in *D. repens* infections of humans in Ukraine, underscoring the growing status of this as a significant emerging infectious disease. The Ukrainian sanitary-epidemiological services managed to achieve some measure of success, one of which is creating a system of registering *D. repens* infections in Ukraine. The mandatory registration of human *D. repens* infections in other European countries would allow to evaluate epidemiological situation in the continent.
FURTHER CASES OF HUMAN DIROFILARIOsis DIAGNOSED IN SOUTH-EAST SERBIA
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Introduction Since the first human infection by Dirofilaria repens was reported in Serbia in 1971, more than 30 cases have been described to date. However, it is well known that clinical cases reported represent only the tip of an iceberg. A recent serosurvey demonstrated a high seroreactivity to dirofilarial antigens in subjects from the northern (27-16%), as well as from eastern and southern Serbia (about 15%).
Here we report molecular confirmation of two further clinical cases diagnosed in 2013 in patients living in south-eastern areas, where human infections have been increasingly reported since the beginning of the century.

Description of the cases A 44-year-old man, living near Leskovac (42°59'N; 21°56'E) (seroprevalence of human dirofilariosis about 8.4%) was admitted to Niš hospital for a subcutaneous nodule in the right infraorbital region. Painless oedema of right cheek skin was the first clinical presentation. Standard laboratory tests did not reveal eosinophilia or any other abnormality. After antimicrobial therapy, oedema was withdrawn but subcutaneous nodule persisted. Then it was surgically removed and a histopathological analysis was performed, which evidenced worm-like structures, whose cuticle with longitudinal ridges permitted the preliminary identification as D. repens-like.

The second patient (57 years old), living in Niš (43°19'N; 21°54'E), the area with lowest seroreactivity to Dirofilaria antigens, consulted an ophthalmologist because of extreme swelling and redness of eyelids, scratches and pain in his right eye. He was treated with antibiotics and corticosteroids and when the swelling was reduced, a mobile parasite in the subconjunctival space was observed. The complete extraction of a living parasite 14cm long was then performed, which was delivered to the Public Health Institute-Niš, where it was identified as a D. repens-like specimen.

In addition in these two patients using a modified Knott test and a commercial filtration test circulating microfilariae in the peripheral blood were not found. Both the paraffin-embedded material and the extracted worm were molecularly processed with D. repens-specific primers. PCR amplification confirmed the diagnosis of D. repens for both clinical cases.

Discussion and conclusions Our reports confirm that human infection by D. repens in Serbia has been increasing steadily, as expected in a Country where the infection rates in dogs is over 60%. Considering this epizootiological status, many human dirofilarial infections could be expected. Continuing education and training of physicians will greatly contribute to the knowledge of the actual impact of dirofilarioses on animal and public health, and allow the planning of suitable measures to prevent these often-neglected infections.
HUMAN DIROFILARIA: FOUR NEW SERBIAN CASES CAUSED BY DIROFILARIA REPENS
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Introduction D. repens is an emerging infectious agent in southeast Europe, currently increasing its geographical range. Human infections are mainly superficial and manifest as either ocular/periocular or subcutaneous lesions. According to parasitological, clinical and serological data in animals and humans, Serbia is endemic region for dirofilariasis and it seems the number of reported cases due to D. repens has gradually increased in the last 15 years. Description of the cases We present four new D. repens infections in Serbia diagnosed from the mid-November 2013 to early April 2014. All the patients were adult females, three were from the City of Belgrade, and one from Jagodina in central Serbia. Three patients have ophthalmic dirofilariasis, two were presented with convoluted worms under the bulbar conjunctiva and one with infection of the external upper palpebra. Subconjunctival infections were accompanied with symptoms of conjunctivitis such as swelling, itching, chemosis and photophobia. Palpebral infection manifested as cutaneous larva migrans with slight redness of the skin. In all three patients intact live worms were extracted by surgery. Patient from Jagodina had subcutaneous mass in the lower abdomen, near the inguinal region initially diagnosed as fibroadenoma. During the summer months she had a few episodes of urticaria unrelated to the location of the lesion which was treated by the general practitioner. The infection was manifested as increasing subcutaneous nodule over a period of approximately six weeks without accompanying symptoms such as pain, pruritus, erythema. The patient only complained of feeling discomfort in the inguinal region and nodule was surgically removed. Eosinophilia was not detected in our patients. In two patients who were tested for microfilariae, no larvae were detected in blood samples. Identification of parasites was based on morphology in direct wet, and histological preparations, and pathohistological sections stained by haematoxylin-eosin and Masson trichrom. In one case, for the reasons of completeness and accuracy, morphological diagnosis was confirmed by PCR. Discussion Ophthalmic dirofilariasis has been increasing in recent years, and according to some reviews, nearly 40% of D. repens-related infections occur in ocular regions. In Serbia, more than 50% of described cases (1971-2014), including this report, had eye infections, mostly subconjunctival. Serious complications such as retinal detachment or other eye damages were not reported in patients from Serbia. The incubation period for human dirofilariasis is approximately 6-8 months and presented patients probably acquired infection during the period from late spring to early fall. Patients with ophthalmic infections had not been abroad during 2013/14. Patient from Jagodina spent two weeks in Netherlands and Belgium in July 2013, so there is some possibility that infection was acquired during vacation, due to reports of D. repens in animals in those countries. Conclusions Human D. repens infection in Serbia is increasing steadily. Infection must be included in the differential diagnosis of various conditions, in a serious way, regardless that most infections are benign and successfully cured by surgery.
NEWER CASE OF HUMAN SUBCUTANEOUS DIROFILARIOSIS IN HUNGARY
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Introduction: Dirofilariosis is an emerging zoonosis in Hungary. We report a case of subcutaneous infection caused by 
Dirofilaria repens, which is a more rarely occurring form in humans.

Description of case: A 74-year-old man presented in the Department of Dermatology. A subcutaneous nodule was seen on his left arm with signs of inflammation. He remembered an insect biting on his left shoulder nearly 2 years ago. Since then a migrating itching area was developed with occasionally appearing nodules on his arm. Repeated inflammations related to physical effects were also detected near the nodules. History of traveling was not mentioned in his anamnesis. An 8-cm long, white worm was eliminated during the surgical intervention. This worm was identified as D. repens according to its morphological features.

Discussion, conclusion: Symptoms of subcutaneous filarioidosis can be appeared within longer time than in ophthalmofilariosis. Surgical intervention can resolve the problem: antiparasitic therapy is not needed in every case. Because traveling to abroad usually are not mentioned in the anamnesis of infected patients, emerging tendency of this zoonosis can be identified in Hungary, too. Thus monitoring of vectors and host animals also plays an important role besides diagnosing and curing the infection correctly.
HUMAN DIROFILARIA REPENS INFECTION IN SOUTHWEST OF IRAN

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Introduction: Dirofilariasis is a common parasitic disease in both domestic and wild animals around the world, with canines as the principal reservoir host and mosquitoes as the vector. *Dirofilaria repens* is prevalent in several regions of the world and because of recent rise in the number of infections in countries of Europe, Africa and Asia, it is considered an emerging zoonosis in these continents. Human *D. repens* infection is reported sporadically from different regions of Iran. The aim of this presentation is report of 6 human cases of dirofilariasis due to *D. repens* from Khuzestan Province, Southwest Iran.

Cases: The patients included five male and one female with the age of 23 to 37 year. The first case was a 34 year old man with a nodule on the right cheek. During the excisional biopsy an adult *Dirofilaria* measured 120 mm in length with white color was removed. The second case was a 37 year old man who noticed a single moveable nodule with itching and irritation and swelling, 15mm in diameter on his chest. The patient pressed the nodule and a white worm with 130mm in length was removed. Third and fourth cases were male and female with the age of 35 and 54 year respectively referred to the ophthalmology department because of irritation with symptoms and signs of itching, swelling and redness of the right eye. Examination noticed living worms in sub-conjunctival space. The worms were white with the lengths of 110 and 105 mm respectively. The fifth case was a young man at the age of 22 year with a nodule on trunk. The nodule thought to be lipoma and during the excision by the surgeon, the white worm was cut in pieces and could not be measured. The sixth case was a 32 year old man with a moveable nodule on his neck with itching, irritation and swelling. During the excision an adult white worm with 180 mm in length was removed. Based on morphological characters and microscopic identified as *Dirofilaria repens*.

Discussion: The development of *D. repens* in unnatural human host is difficult and only rarely subcutaneous containing gravid female worms have been described. Infections in humans are usually asymptomatic and noted only when worms enter the conjunctiva as mentioned in our cases. None of the parasites isolated from our cases developed to mature worms.

Conclusion: Increasing of human dirofilariasis may be attributed to environmental changes with global warming, humidity and increase of mosquito vectors and breeding, agricultural development and change in social conditions, traveling and outdoor living.
DIRECT PCR FOR DETECTION OF DIFFERENT DIROFILARIA SPP. AND STAGES IN DIFFERENT SUBSTRATES
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Introduction
The detection of filarioid worms, e.g. *Dirofilaria* spp., in blood, vector or tissue is of great importance for risk assessment, epidemiology, treatment and counter measures for parasite control. The differentiation of microfilariae based on morphological parameters is possible, but PCR is preferred due to its greater sensitivity and accuracy. Until now, PCR preparation consequently requires DNA preparation, which is time- and cost consuming. We therefore developed a novel direct PCR technique for rapid and straightforward detection of filarial DNA in various substrates.

Methods
A novel approach omitting a prior extraction step, using a different polymerase during the PCR, was carried out in this study. This PCR was conducted for L1 microfilaria (mf) of *D. repens* and *D. immitis* in EDTA blood stored on FTA filter paper, for EDTA blood and for Knott test material. Furthermore, mosquito (L3) artificially infected with *D. immitis* and pieces of an adult *D. immitis* worm, as well as formalin-fixed skin nodules containing *D. repens* adults were used. Primers targeting a 667 bp fragment of the COI gene were chosen with an adapted protocol for the “direct PCR” polymerase.

Results
Mf of *D. repens* and *D. immitis* on the filter paper, L3 in the mosquito and worm fragments of *D. immitis* displayed a good response in the PCR. The EDTA blood, Knott test material and the formalin treated tissue did not reveal any positive PCR result.

Discussion
While the direct PCR method is sufficiently sensitive and robust to detect mf on filter paper, L3 larvae in mosquitoes and adult pieces of worms, substrate with inhibitory contents (EDTA in blood) or degraded DNA (e.g. by formalin) was unsuitable for amplification in direct PCR. Therefore processing these substrates with the direct PCR method – as with conventional PCR - has to be exercised with caution.

Conclusion
This technique is a rapid and powerful tool to investigate several substrates for different stages of *Dirofilaria* spp. parasites. The method could be adapted to investigate other filarioid worms and can be used for high-throughput processing of samples.
THE USE OF SCREENING BLOOD-FED MOSQUITOES FOR THE DIAGNOSIS OF 
DIROFILARIA

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Introduction
Both Dirofilaria repens and D. immitis are known to be endemic in Hungary. A fatal case of a 
dog infested with D. immitis received attention from the media and there were other cases, 
too, so it was decided to catch mosquitoes in the garden where the dog lived to screen for 
filarioid nematodes with molecular tools.

Methods
Mosquitoes were caught in a garden in Szeged (Southern Hungary) with M-360 electric 
mosquito traps and were stored in ethanol until further processing. Female mosquitoes were 
classified to genus level only (by morphology). Each mosquito was homogenized and 
analyzed for filarioid helminths and avian malaria using standardized PCR techniques. 
Positive mosquito samples were further specified to species level with barcoding (COI gene).

Results
More than 250 blood-fed mosquitoes were caught in the garden of the infected dog. 
Molecular screening revealed that not only D. immitis was present in the analyzed specimens. 
DNA of D. repens, Setaria sp. and Plasmodium sp. was confirmed as well.

Discussion
The analysis of blood-fed mosquitoes for the diagnosis of Dirofilaria sp. and other mosquito 
borne pathogens seems to be an adequate technique to evaluate if filarioid helminths are 
present in a certain area. Normally only unfed female mosquitoes are analyzed for 
epidemiological studies. However, blood-fed mosquitoes can only be used for screening if a 
pathogen is present because the role of the mosquito as vector cannot be classified (blood of 
bitten host).

Conclusion
The screening of blood-fed mosquitoes for filarioid helminths is an adequate tool to confirm 
the presence of Dirofilaria sp. in a certain area.
MICROANATOMICAL COMPARISON OF HUMAN AND ANIMAL DIROFILARIA REPENS ISOLATED IN IRAN

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Introduction
Dirofilariasis is a zoonotic filarial parasitic infection. Both human and animal dirofilariasis due to Dirofilaria immitis, and D. repens have been reported from different parts of Iran.

Objectives
Different organs are infected with D. repens with different clinical symptoms. Biopsy is useful for diagnosis; however, this could be misdiagnosed with other infections and tumors as a small part of the parasite may be observed in biopsied specimen and microfilariae usually fail to complete maturation in human cases. In histopathological evaluation of human cases only a small part of the worm may be observed which makes it difficult for thorough diagnosis. So, micro-anatomical study of D. repens using serial sections for comparison of human cases is very useful for a more reliable diagnosis in tissue infections.

Methods
An adult D. repens originated from a subcutaneous infection of a dog was serial sectioned and compared with sections of D. repens in a nodule of human removed by surgery. Specimens were fixed in formaldehyde followed by paraffin embedding and sectioning using microtome. The sections were stained and mounted. The micro-anatomical structure of both human and animal D. repens were compared and different photos were made.

Results
Histologic sections of human D. repens showed transverse sections of a female worm with a diameter of about 408 × 265 micrometer. The worm had a thick cuticle with coarse external longitudinal cuticular ridges, prominent lateral cords and prominent somatic musculature. Paired uteri contain many microfilariae. The surrounding tissue showed acute and chronic granulomatous inflammation with giant cells and eosinophils. On the other hand morphologic evaluation of cross and longitudinal sections of animal D. repens showed an average diameter of 500 micrometer with multilayer thick cuticles, indistinct intestinal cells, numerous external ridges, distinct dorsal and ventral fields of divided coelomyarian somatic musculature, two internal longitudinal ridges with broad lateral chords, heavy musculature, and didelphic uterine tubes with a small extra branch full of microfilariae presenting the characteristics of mature female worm of D. repens.

Discussion and conclusions
D. repens causes different clinical manifestations in human. Usually, physicians and pathologists are not familiar with dirofilariasis infections; moreover in pathology sections the whole worm could not be observed and only a small part of the worm may be observed; makes it difficult for a reliable diagnosis. So, study on microanatomy of whole worm with animal origin which demonstrates more details of the worm is very useful for comparing the histopathological sections of the human cases even if a small part of the worm is visible.
MOLECULAR DIAGNOSTICS OF SOME MICROFILARIA SPECIES IN UPPER EGYPT REGION

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Introduction
Molecular techniques are utilized for the diagnosis of parasitic diseases and identification of parasites, for the development of specific antigens for serological tests and studying immune response in the patients.

Objectives
In the present study, Wuchereria bancrofti from man, Dirofilaria species from man and dogs in upper Egypt region

Material and methods
All samples were checked with 3 different primers by the use of RAPD-PCR. The genetic profiles of all spp. from different hosts were compared. Pairwise comparisons were used to evaluate sequence homology and diversity of some variable regions were identified. Interspecific variation in the regions exceeded that within species. Comparison between Dirofilaria from man and that from dogs revealed genetic polymorphism with genetic variability observed in DNA amplification with primer 3 and non-genetic polymorphism with genetic variability in primer 1 and primer 2.

Conclusion
The use of the Random Amplified Polymorphic DNA - Polymerase chain reaction (RAPD-PCR) technique to amplify short regions of an organism’s genome provide more specific method than conventionally employed in epidemiological studies.
MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE PLASMINOGEN-BINDING PROTEINS OF DIROFILARIA IMMITIS ACT, FBAL, GAPDH AND GAL

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Introduction
Fibrinolysis is one of the main antithrombotic mechanisms of the hemostatic system. Blood plasminogen binding to specific receptors and tissue plasminogen activator (t-PA) leads to their conversion into plasmin, enzyme responsible for removing clots. In order to survive and propagate into the circulatory system, blood-borne pathogens should avoid blood clotting through interaction with the fibrinolytic system. During cardiopulmonary dirofilariosis \textit{D. immitis} adult worms living for years in pulmonary arteries and right ventricle of the infected hosts cause chronic inflammatory pathology and thromboembolisms. Therefore, it seems reasonable to assume that \textit{D. immitis} uses its metabolic products to interact with its host fibrinolytic system. In previous studies, we have demonstrated the interaction between two antigenic compartments of \textit{D. immitis} (excretory/secretory and surface) and the fibrinolytic system, identifying 15 plasminogen-binding proteins from the parasite.

Objectives
In this context, the aim of this work was to produce four of these molecules [actin (ACT), fructose-bisphosphatealdolase (FBAL), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and galectin (GAL)] as recombinant proteins in order to analyze their ability to activate the fibrinolytic system and to demonstrate their expression on the host-parasite interface.

Methods
After designing the primers of the proteins, the genetic material was amplified and isolated. DNA fragments were inserted in a PSC-A cloning vector, and the obtained sequences were developed in their recombinant form in the TOPO/pDEST expression system. Once purified, the capacity of the proteins to bind plasminogen and generate plasmin was studied by ELISA in the presence/absence of t-PA. The anatomical localization of the proteins was carried out in histological sections of adult worms by immunofluorescence using rabbit polyclonal sera against parasitic proteins.

Results
The four recombinant proteins bind plasminogen and generate plasmin. This binding was directly proportional to the amount of plasminogen and none of them was able to generate plasmin in the absence of t-PA. Microscope images showed the distribution of the four proteins by all the soma of the parasite, being especially abundant in the cuticle.

Discussion
The results suggest that ACT, FBAL, GAPDH and GAL of \textit{D. immitis} interact with the fibrinolytic system of the host. In addition, the localization of the proteins as components of the host-parasite interface would facilitate their participation in the activation of this system.

Conclusions
These findings suggest that \textit{D. immitis} adult worms could control clots development as a way to facilitate their survival in the circulatory system of the host.
CAN THE ACTIVATION OF PLASMINOGEN/PLASMIN SYSTEM OF THE HOST BY METABOLIC PRODUCTS OF DIROFILARIA IMMITIS PARTICIPATE IN HEARTWORM DISEASE ENDARTERITIS?

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Introduction Dirofilaria immitis is the causative agent of animal and human dirofilariosis. Dogs are considered the definitive hosts and the adult worms of D. immitis can survive for years in their pulmonary arteries and the right heart chambers causing a chronic inflammatory pathology at vascular level. One of the key pathological events is the appearance of proliferative endarteritis. It has been described that this process is accompanied by the proliferation and migration of the cells of the arterial wall, as well as the destruction of the extracellular matrix. On the other hand, despite the fact that an acute pathology with formation of serious thromboembolism can occur, D. immitis has the ability to regulate these pathological mechanisms contributing to its survival in the intravascular habitat. We have recently demonstrated that the excretory/secretory antigens from D. immitis adult worms (DiES) interact with the host fibrinolytic system binding plasminogen (PLG) and generating plasmin. Plasmin is the enzyme responsible for the lysis of fibrin clots. Nevertheless, the resulting maintenance of hemostasis (a priori beneficial for both the parasite and host) could have pathological consequences. In cardiovascular research, an overstimulation of the plasminogen/plasmin system has been related to the proliferation and migration of the cells of the human arterial wall and with the extracellular matrix degradation.

Objectives The aim of this work is to demonstrate that the overproduction of plasmin by DiES causes proliferation, migration and intracellular matrix degradation in canine endothelial and smooth muscle cells.

Methods Cell proliferation through the crystal violet technique, cell migration by wound healing assay and degradation of the extracellular matrix by measuring expression of matrix metalloproteinases and collagen degradation were studied in an “in vitro” model using canine vascular endothelial and smooth muscle cells. These cells were treated with a mixture of DiES+PLG. Untreated cells, cells only stimulated with DiES or with PLG, or with a mixture of DiES+PLG+εACA (an inhibitor of the plasminogen-plasmin conversion) were employed as controls.

Results In all experiments, statistically significant differences between DiES+PLG and control groups were obtained.

Discussion and Conclusion Results have highlighted the DiES participation in proliferation, migration, and degradation of the extracellular matrix of canine endothelial and smooth muscle cells via plasminogen/plasmin system activation. This fact suggests that the overproduction of plasmin as a survival mechanism of D. immitis may have also pathological implications at vascular level by its participation in the stimulation of all the processes related to proliferative endarteritis.
P18
CERCOPITHIFILARIA BAINAE IN A DOG SUFFERING FOR CHRONIC POLYARTHRITIS
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5Private practice, Ambulatorio Veterinario Associato “Bufalini e Giannini”, Viterbo, Rome, Italy. 6Novartis Animal Health, Origgio, Varese, Italy.

Introduction Filarioids of dogs are attracting attention of parasitologists across Europe because of the risk of their spread into previously non-endemic areas and as emerging zoonotic agents. The species of major impact on veterinary medicine and public health are Dirofilaria immitis and Dirofilaria repens; however, other filarioids of the genus Cercopithifilaria Eberhard, 1980 (Spirurida, Onchocercidae), as C. bainae, C. grassii and Cercopithifilaria sp. II sensu Otranto, have been recently described in dogs. In spite of its broad geographic distribution, which overlaps that of its main vector, the brown dog tick Rhipicephalus sanguineus s.l., C. Bainae is considered of minor impact in veterinary medicine. Indeed, to date, only a single case of dermatological alteration has been reported in an infected dog. Herein we report a case of chronic polyarthritis in a dog infected by C. bainae whose microfilariae were found in the inflamed joints.

Description of the case A 7-year old mixed breed dog living in Viterbo (Latium, Central Italy) was admitted to a private practitioner with a history of walking reluctance, laziness and lameness. At the clinical examination, the dog displayed multiple joint pain during the manipulation of both thoracic and the pelvic limbs. A diagnosis of chronic polyarthritis was issued and the dog was unsuccessful treated with two anti-inflammatory cycles. Biochemical and haematological analyses were within normal ranges and no alterations were found at the x-ray examination. Therefore, synovial fluid from joints was collected, whose examination by microscopy evidenced alive and actively moving microfilariae, which were morphometrical identified as C. bainae based on their measurements and features. Genomic DNA was then extracted from both microfilariae collected and a skin sample, and the partial cox1 gene fragment (~304 bp) from Cercopithifilaria spp. was amplified. Sequence analysis confirmed the molecular identification of C. bainae haplotype I (GenBank AN: JF461457).

Discussion Although the location of C. bainae adults and the microfilariae presence in the host body has been recently assessed, the unexpected additional presence of microfilariae in the synovial fluid could be considered an aberrant location due an inflammatory status. However, this unusual localization would also suggest a possible involvement of C. bainae in the occurrence of these lesions. Further studies are needed in order to elucidate the real pathogenic potential of this parasite, as well as effective pharmaceutical ingredients and treatment schemes should be evaluated for the prevention and management of this little known but widely distributed filarioid of dogs.
STUDIES ON DIROFILARIA REPENS AND ACANTHOCHEILONEMA RECONDITUM IN STRAY DOGS IN ERZURUM PROVINCE, TURKEY

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Ataturk University, Faculty of Veterinary Medicine, Department of Parasitology, Erzurum-Turkey

Introduction
Filarioidosis in dogs is caused by several species of filarioids. From these, Dirofilaria immitis, D. repens and Acanthocheilonema reconditum have been reported from dogs in various parts of Turkey. The importance of the infection in veterinary medicine and public health, makes it essential to carry out epidemiological studies and determine the prevalence of the agents. This study was conducted in order to investigate the prevalence of D. repens and A. reconditum species in Erzurum province of Turkey in stray dogs that did not undergo prophylaxis for filarioidosis.

Materials and methods
Between April 2013 and April 2014, 133 (28 male, 105 female) stray dogs were sampled. Of the sampled dogs, 41 (30.8 %) were 0.5-1 years old, 65 (48.9 %) were 1-3 years old and 27 (20.3 %) were 3-8 years old. For molecular identification, species specific primers were used to amplify fragments of the cytochrome oxidase subunit 1 gene of A. reconditum (208 bp) and D. repens(209 bp).
None of the samples showed a positive reaction to D. repens or A. reconditum by PCR test.

In conclusion, the prevalence and epidemiology of vector-borne diseases in different regions is variable depending on the climatic conditions, vector and host populations. As a result, it was concluded that dog filarioidosis caused by D. repens and A. reconditum is not a serious health concern in Erzurum, Turkey.

The study protocol was approved by the Animal Care and Use Committee at Ataturk University 2012/50 decision number).
ANGIOSTRONGYLUS VASORUM IN STRAY DOGS IN THE CITY OF NAPLES, SOUTHERN ITALY
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Introduction  Cardio-pulmonary nematodes, as Angiostrongylus vasorum, are spreading in dogs and can be responsible for severe clinical forms. For these reason they are considered very important pathogens in the clinical practice of these companion animals, therefore appropriate techniques for diagnosis and regular monitoring of these parasites are considered fundamental to reduce the risk of infection in dogs.

The objective: The present study reports the results of a survey on the presence and distribution of A. vasorum in stray dogs in the city of Naples (southern Italy).

Materials and methods  From 2009 to 2013, faecal samples were routinely collected from 2828 stray dogs living in the city of Naples and brought to the veterinary hospital of the Department of Veterinary Medicine and Animal Productions, University of Naples “Federico II”, for sterilization. Information regarding sex, age and clinical signs was collected at the time of arrival to the veterinary hospital. Microscopic coprological examination employed the FLOTAC basic technique having an analytic sensitivity of 1 larva per gram of faeces (LPG). A zinc sulphate flotation solution (FS3, specific gravity = 1.20) was used to identify and count the L1 of A. vasorum.

Results and Discussion  The results of the survey showed a prevalence of 0.5% (n. of positive dogs=14). The results stratified according to sex, size and age are shown in Table 1.

Table 1 - Number of dogs stratified according to sex, size and age and prevalence (%) of A. vasorum.

<table>
<thead>
<tr>
<th></th>
<th>n. of dogs</th>
<th>prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1439</td>
<td>0.4</td>
</tr>
<tr>
<td>Female</td>
<td>1389</td>
<td>0.7</td>
</tr>
<tr>
<td>Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>766</td>
<td>0.5</td>
</tr>
<tr>
<td>Medium</td>
<td>1646</td>
<td>0.1</td>
</tr>
<tr>
<td>Small</td>
<td>416</td>
<td>0.2</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 8 months</td>
<td>672</td>
<td>0.3</td>
</tr>
<tr>
<td>&gt; 8 months</td>
<td>2156</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Statistical analysis was carried out using STATA 10.0 software (Stata Corp., Texas 77845, USA). No significant differences were observed regarding sex, size and age of the animals using Pearson’s Chi squared test for independence (P>0.05). Unfortunately, there are few similar studies on stray dogs so it is difficult to compare the prevalence found in the present study with the values found in other studies also due to the different methods used for diagnosis; however, our results are similar to those observed in dogs shelters in central and southern Italy. In conclusion, considering the results of the present study, it is desirable to plan such parasitological investigations also on a large scale, using appropriate diagnostic methods in order to better target effective control strategies against A. vasorum.
NEW EPIDEMIOLOGICAL DATA ON ANGIOSTRONGYLUS VASORUM DISTRIBUTION IN GERMANY

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\textbf{Introduction}

Infections with \textit{Angiostrongylus vasorum} can cause severe cardiopulmonary diseases in dogs occasionally being associated with coagulopathies, neurological disorders or even death. In the past, canine angiostrongylosis has largely been neglected in Europe although several recent studies have proven an expansion of the geographic distribution of the disease, a phenomenon that was also reported for the closely related lungworm \textit{Crenosoma vulpis}.

The \textbf{aim} of the present study was to combine our recent data on \textit{A. vasorum}- and \textit{C. vulpis} infections in German dogs covering the years 2003 to 2007 with new results from the years 2008 to 2013 to calculate expanding tendencies for \textit{A. vasorum} and \textit{C. vulpis} occurrence frequencies.

\textbf{Materials and Methods}

In total 7660 faecal samples from German dogs displaying clinical signs were analyzed by the Baermann funnel technique for the presence of \textit{A. vasorum} and \textit{C. vulpis} larvae.

\textbf{Results}

In total, \textit{A. vasorum} and \textit{C. vulpis} larvae were found in 160 (2\%) and 138 (1.8\%) samples, respectively. Preliminary results reveal that geographically, \textit{A. vasorum} and \textit{C. vulpis} infections were found to be widely spread in Germany. They were present in 12 and 14 out of 16 German Federal States, respectively.

\textbf{Discussion and conclusion}

The epidemiological analysis of the current data revealed that the occurrence of \textit{A. vasorum} infections was, by trend, declining in all ‘positive’ Federal States, with the exception of Baden-Wuerttemberg. In contrast, an increase of the \textit{A. vasorum} occurrence from 2.5\% to 4.2\% within the years 2003 to 2013 was estimated for Baden-Wuerttemberg. In the case of \textit{C. vulpis}, the number of positive samples decreased in 12 German Federal States and only in Saxony and in Mecklenburg-West Pomerania an increase from 0\% to 6.6\% and from 0\% to 12\% was observed, respectively.
P22

ANGIOSTRONGYLOSIS - CURRENT SITUATION IN PORTUGAL
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Introduction
Angiostrongylus vasorum is considered by several authors as an emergent parasite due to its apparent worldwide recent spread. It is a potentially lethal parasite living in the heart and pulmonary arteries of domestic and wild animals.

Objective
The present work aimed to update the recent cases of A. vasorum infection, gathering all the available data, together with their geographical dispersion and its host distribution in Portugal.

Methods and results
The first necropsy study was performed in red foxes (Vulpes vulpes silacea), from 1970 to 1987, in the coastal central and south regions of Portugal, nearby Tejo and Sado basins, revealing an occurrence rate of 0.3% (1/306) for A. vasorum infection. From 2000 to 2006, 16.1% (10/62) of the red foxes collected in Coimbra (Dunas de Mira), littoral centre of Portugal, showed A. vasorum nematodes in necropsy. The last study performed in red foxes occurred in 2010/2011, in Elvas, Alentejo, revealing an occurrence rate of 1.8%(1/55) for A. vasorum first-stage larvae using Baermann test coprology. Additionally in 2010, one case of A. vasorum infection was reported during the necropsy of a 14-year-old male red panda Ailurus fulgens fulgens from the Lisbon Zoological Park. Regarding angiostrongylosis in domestic carnivores, three positive cases were found in the Lisbon area in the last few years: in 2006, a two-year-old, male, King Charles Spaniel was presented with haemorrhagic gastroenteritis and severe dyspnoea, revealing two adult nematodes inside the lumen of bronchiolar arterioles, in the post-mortem examination; in 2012, an adult mixed-breed male dog with a history of weight loss showed first-stage larvae in Baermann test; similarly, in 2013, a 13-year-old, mixed-breed male dog presented with diarrhoea and weight loss was diagnosed with angiostrongylosis using Baermann test, not showing any respiratory, neurological or coagulopathic signs. In 2014, the first serological detection of circulating A. vasorum antigen and parasite-specific antibodies was conducted in shelter dogs from the centre-districts of Portugal (Coimbra, Santarém and Setúbal). Of the total canids analysed, 1.2% (4/341) were positive for both A. vasorum antigen and antibody, showing an active infection. Also 2.4% (8/341) were A. vasorum antibody-positive, enhancing a previous exposure to this parasite, in the three surveyed regions.

Discussion and conclusion
All this several reports from the centre and south of Portugal aimed to alert veterinarians and owners of angiostrongylosis cosmopolitan distribution in domestic and wild reservoirs, as well as, its challenging variety of clinical manifestations, occasionally even asymptomatic.

Funding: Portuguese Foundation for Science and Technology through a PhD research grant SFRH/BD/85427/2012. This work was done under the frame of EurNegVec COST Action TD1303.
P23
EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF ANGIOSTRONGYLUS VASORUM IN ITS INTERMEDIATE HOSTS, AND IMPLICATIONS FOR TRANSMISSION AND SPREAD IN EUROPE
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Introduction
The development of Angiostrongylus vasorum in three gastropod species was investigated, with a particular emphasis on the role of temperature in development. These species were: The aquatic, planorbid snail Biomphalaria glabrata and two UK endemic terrestrial slug species representing two different slug families; the arionid slug Arion hortensis and the Limacid slug Limax marginatus.

The main aim was to determine an estimate of threshold temperature (T₀) for development of A. vasorum and to gain an understanding of the duration required at temperatures above T₀ for development to the infective L3 stage to occur.

Results
Development rate was found to be strongly dependent on temperature, with a lower predicted threshold of 5 °C, and a time of development from L1 to L3 ranging from 74 days at 10 °C to 18 days at 25 °C. Development success decreased with increasing temperature and varied between gastropod species. The predictions were tested in the field in spring 2014, to determine whether overwintering of larvae in slugs could be contributing to emergence of angiostrongylosis in endemic areas. Of 200 slugs of mixed arionid and limacid species collected in March 2014, none were positive by Baermann’s examination, in agreement with low prevalence (3%, n = 146) by qPCR in a previous study in February 2010. By comparison, 21-55% of slugs across months and species (n = 569) were positive between May and October in previous years.

Conclusion
Results suggest that over-winter persistence of A. vasorum in slug populations is epidemiologically unimportant. Further studies are needed.
In Switzerland, first cases of Angiostrongylus vasorum infections were reported from a dog breeding station in Zurich in 1968. Only recently, infected dogs and also foxes were diagnosed from the northern and southern parts of Switzerland.

Objective
Because further emergence of canine angiostrongylosis, epidemiological investigations on A. vasorum in foxes were initiated in order to assess the risk of infection for the Swiss dog population.

Materials and Methods
A total of 1373 fox faecal samples were collected applying a standardized and reproducible field method using GPS (Garmin, GPSMAP® 60CSx). All in all, 2145 km transects in totally 171 one square kilometer areas throughout Switzerland were investigated between August 2010 and January 2012. Faecal analysis was performed as previously described for the isolation of eggs of Echinococcus multilocularis with modifications, and first stage larvae were identified based on morphological structures using an inverted light microscope (Labovert FS, Leitz Wetzlar, Germany). Biomolecular analyses were performed to confirm the presence of A. vasorum-DNA and to test questionable samples containing morphologically unidentifiable larvae.

Results
Totally 115 samples (8.4%) were identified positive for A. vasorum. Samples collected below 400 m, between 400-700 m and above 700 m above sea level were A. vasorum positive in 20.6% (41/199), 8% (71/887) and 1.1% (3/287), respectively. Positive samples were collected in both agricultural and urbanized areas from all bioregions of the country. Additionally, a total of 199 culled foxes from the extended area of Zurich were used to evaluate ELISAs, which were developed for detecting circulating antigens of A. vasorum or specific antibodies in dogs, for their use with fox samples. Overall, necropsy prevalence in these foxes was 26.3%, with seasonal differences and worm burdens ranging from 1-30. Both ELISAs were applicable with blood samples from foxes, and in particular sensitivity and specificity of the antigen ELISA were high.

In conclusion, serological assays were suitable for detecting A. vasorum infectionsin blood samples of foxes and can be used for mass-screening of fox populations instead of more laborious dissection methods, while the use of a standardized field method for collection of fox faecal samples and correspondingly adapted laboratory procedures are recommended to assess the abundance of A. vasorum in areas where foxes are not hunted.
Efficacy of a 10% Imidacloprid/2.5% Moxidectin Spot-on Formulation in the Treatment of Natural Nasal Eucoleosis in Dogs

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³Bayer Animal Health GmbH, Leverkusen, Germany

Introduction
Capillaria boehmi (syn. Eucoleus boehmi) is a nematode inhabiting nasal turbinates and sinuses of domestic dogs and wild animals. No drug is presently licensed for the treatment of nasal capillariosis in dogs, thus the therapeutic regimens attempted so far have been derived empirically.

Aim
With the aim to fill these gaps, a pilot trial was performed to investigate the efficacy and safety of a spot-on formulation containing 10% imidacloprid and 2.5% moxidectin (Advocate®, Bayer Animal Health GmbH, Leverkusen, Germany) in the treatment of the infection by C. boehmi in dog.

Materials and methods
Sixteen dogs copromicroscopically positive for C. boehmi eggs were confirmed, either by rhinoscopy or species-specific PCR-coupled sequencing assay, as being affected by nasal capillariosis and thus enrolled for the trial. The animals were randomly allocated to 2 different study groups: Group T, treated on Day 0 with Advocate®, and Group C, left untreated, in a ratio of 1:1. The animals underwent clinical examination and quantitative copromicroscopy using a McMaster technique on Days -6 and -2 (baseline) and Day 28 ± 2 (post-baseline). On Day 28 ± 2 the efficacy of the treatment or the persistence of the infection were confirmed by rhinoscopy or, in alternative, by molecular procedures. Dogs in Group C which were still infected on Day 28 ± 2 received a rescue dose of Advocate® and were re-examined on Day 56 ± 2.

Results
No adverse effects were recorded in any of the treated dogs. Seven of the 8 dogs in Group T were negative on Day 28 ± 2 (reduction of baseline faecal egg counts by 99.14%), while one dog resulted negative at Day 56 ± 2 after a second treatment on Day 28 ± 2 (reduction of baseline faecal egg counts by 100% in Group T). Seven animals in Group C received a rescue dose of Advocate® on Day 28 ± 2 and scored copromicroscopically and molecularly negative on Day 56 ± 2, thus increasing the reduction of post-baseline egg counts to 99.57% after a single administration. A fully recovery of clinical signs in seven of the eight symptomatic animals of the Group T and in five animals which received the rescue treatment was obtained 4 weeks after the first treatment given.

Discussion and conclusions
These promising results showed that a single administration of Advocate® is a safe and effective option for treating clinical signs and eliminating egg shedding and in situ adult parasites in dogs infected by C. boehmi under field conditions.
Introduction

Angiostrongylus vasorum is a globally distributed nematode that causes severe clinical signs in dogs. Despite the widespread distribution of canine angiostrongylosis and its clinical importance, this disease is often underestimated, likely for the lack of information on the epidemiology of A. vasorum and for the drawbacks inherent to the clinical and copromicroscopic diagnosis. Clinical signs of dog angiostrongylosis are aspecific and the Baermann’s method, i.e. the gold standard technique for the aetiological diagnosis, is not commonly performed in clinical practice.

Materials and Methods

The present study described cases of asymptomatic angiostrongylosis in a breeding kennel of Italy, alongside the evaluation of the efficiency of a newly marketed rapid kit (IDEXX Angio Detect™ Test) for the field diagnosis of the disease before and after an anthelmintic treatment with a parasiticide formulation licensed for the treatment of A. vasorum infection. The study has been carried out in a kennel of Jack Russell Terrier located in central Italy with a recent history of angiostrongylosis. At Day -15 the fifteen dogs bred in the kennel were clinically examined and subjected to a copromicroscopic examination and to the rapid kit Angio Detect™ Test. Animals (n. 3) which scored positive at least at one diagnostic assay were again tested with the Baermann’s and Angio Detect™ Test at Day 0 and then treated with a spot on formulation containing moxidectin 2.5%/imidacloprid10% (Advocate®, Bayer).

Results

Two and four weeks post treatment the treated dogs scored negative at both tests. At Days -15 and 0 three dogs scored positive for A. vasorum L1s and two of them also at the rapid kit. Two and four weeks after the treatment with Advocate®, they scored negative at both tests. The animals were asymptomatic throughout the study period.

Discussion and Conclusion

The present study confirms that A. vasorum is present in certain areas of Italy, where it can cause both asymptomatic and symptomatic infections. The absence of symptoms in the herein examined dogs underlines the need to periodically perform diagnostic tests in dogs living in endemic areas, regardless the presence of clinical signs compatible with the infection. The negativity of one dog at the kit although positive at the Baermann’s test may be accounted for an early stage of the infection, as supported by the lack of symptoms. Considering the low number of positive dogs in the present study, further studies are necessary to evaluate more in depth the concordance between the two methods in the field. Finally, this study confirms that Advocate® spot on is highly effective in treating dog angiostrongylosis.
FIRST CLINICAL CASE REPORT OF CANINE ANGIOSTRONGYLOSIS DIRECTLY DIAGNOSED IN POLAND

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3Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine University of life sciences, Lublin, Poland

Introduction
The prevalence of dog's angiostrongylosis in eastern European countries is poorly known. So far little is known about its presence in Poland. Seroprevalence of angiostrongylosis in dogs from different parts of Poland in 2012 was 0.51%. This article is the first report describing a clinical case of canine angiostrongylosis directly diagnosed in Poland.

Description of the case
The authors present a clinical case of infestation with *Angiostrongylus vasorum* in a 1.5-year-old female dog of the Dalmatian breed. Different clinical symptoms including coughing, emesis with blood, and occasional bleeding from the nostrils were observed. Its diagnosis was based on radiological and endoscopic examinations. A thoracic radiograph revealed a moderate decrease of lung tissue opacity, which was intensified on peripheral parts of caudal lung lobes. The decreasing of radioopacity was due to diffuse parenchymal and peribronchial infiltration. There was also visible a moderately right ventricle enlargement. A major pulmonary vessels appeared normal. A contrast examination allowed for the exclusion of the esophagus perforation. During a bronchoscopy increased volume of mucus with a tiny little streak of blood was visualized. Morphological and biochemical examinations of the blood were also performed. A definite diagnosis was made by parasitological investigation. A characteristic morphological details of L1 larvae were visualized using a differential interference contrast (DIC) microscopy. The larvae were observed in stool samples and in samples obtained using standard endoscopic instruments. Fecal samples collected from the rectum were tested using flotation with solution saturated NaCl and sucrose (specific gravity 1.25). Treatment was successful, and all alarming symptoms subsided.

Discussion and conclusion
The prevalence of *Angiostrongylus* seems to be underestimated. It is believed that a precise diagnosis is difficult because of Baermann technique is not often performed in parasitological investigation of dogs. Described case demonstrates that the larvae may be found in stool samples also using flotation technique with solution saturated with specific gravity 1.25. Furthermore during diagnosis procedures, bronchoscopy and radiology may be useful in the clinical course of angiostrongylosis.
CUTANEOUS LARVA MIGRANS DUE TO ANGIOSTRONGYLUS VASORUM IN A DOG
Paola Cavana¹, Aurélien Jaendel¹, Emmanuel Bensignor¹, Stéphane Blot¹, Odile Crosaz¹, Marine Carlus¹, Nathalie Cordonnier¹, Peter Deplazes², Bruno Polack¹
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Introduction
Angiostrongylus vasorum is a nematode classically responsible for respiratory disease, bleeding disorders and miscellaneous disease like neurological or gastro-intestinal signs in dogs. Skin lesions associated with this parasite are very unusual.

Objective
We report here one case of cutaneous larva migrans due to this parasite.

Description of the case
A 3 years old female Weimaraner was referred for acute lesions on the nose, ear pinnae and one foot. The dog was otherwise healthy but was under long term treatment with corticosteroids and azathioprine for an aseptic meningitis since one year. Clinical signs included erythema, alopecia, papules and raised plaques with hyperkeratosis on the bridge of the nose and the ear pinnae and alopecia, swelling and perionyxis on one foot. No pruritus was reported. Biochemistry was unremarkable except for increased ALP. CBC showed a mild leucocytosis without eosinophilia. Histopathological exam demonstrated numerous dermal pyogranulomas with eosinophils centered around parasitic elements (250-300 µm long) compatible with larvae of A. vasorum. The presence of A. vasorum was confirmed by in situ PCR with specific primers. A Baermann’s test demonstrated the presence of numerous larvae of A. vasorum. Coproscopic examination demonstrated also the presence of eggs of Uncinaria stenocephala and Eucoleus boehmi. A few days later the dog developed respiratory distress and lethargy. Chest radiographs showed an alveolar and interstitial opacity compatible with angiostrongylosis.
The dog was treated with fenbendazole 20 mg/kg/day for 3 weeks. A marked improvement of skin lesions was reported as soon as 5 days after the first dose.

Conclusion
To the best of our knowledge this is the first case of a dog infested with larvae of A. vasorum presented initially with only skin lesions.
CANINE PULMONARY ANGIOSTRONGYLOSIS, AN UNUSUAL RESPIRATORY DISORDER FOLLOWED BY ARTERIAL BLOOD GAS ANALYSIS

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Introduction
Pulmonary angiostrongylosis was diagnosed in a young dog which was hospitalized in the Small Animal Hospital of the Faculty of Veterinary Science, Szent István University. Angiostrongylus vasorum infection was proved by Baermann technique from bronchoalveolar lavage and fecal samples. Restrictive pneumopathy was detected and followed by serial arterial blood gas analyses.

Description of the case
A five-month-old male, mongrel dog was admitted to the Intensive Care Unit with a few-weeks-long history of dyspnea and coughing. Marked eosinophilia and bronchopneumonia were detected. Pulmonary angiostrongylosis was determined by Baermann method and larval identification. Eleven-days-long fenbendazole (50mg/kg SIDpo) cure was carried on. Repeated parasitological evaluation of the fecal sample (collected on three consecutive days) and bronchoalveolar lavage sample did not reveal persistent A. vasorum infection. Control hematology, thoracic radiographs, bronchoscopy and blood gas analysis were performed to follow the course of the illness. The clinical symptoms eased within a short time, but the ongoing ventilation disorder was assessed by arterial blood gas analysis. The femoral artery was punctured to obtain arterial blood sample and venous sample was collected from saphenous vein at the same time without anaesthesia. The venous pCO2-level showed a mild increase (51.7 mmHg) initially then it returned to the reference interval (32-49 mmHg). The venous pO2 level varied between 30.2-56.1 mmHg (ref. range: 24-48 mmHg) irrespectively of the severity of clinical symptoms. While the arterial pCO2 values were physiologic 36.6-39.9 mmHg (ref. range: 36-44 mmHg) in every occasions the arterial pO2 level increased from the initial 75.6 mmHg to 92.3 mmHg (ref. range: 90-100 mmHg). The lowest arterial pO2 level was measured after the beginning of fenbendazole administration and eosinophilia got more severe simultaneously.

Discussion
Restrictive pneumopathy was concluded based on the arterial blood gas levels. The lowest pO2 level could be explained by local inflammatory process triggered by the dying/dead lungworms in the pulmonary tissue due to the anthelmintic treatment. The elevated number of eosinophils in the blood is in accordance with this result. To reduce the inflammation prednisolone (1mg/kg SID po) was add to the therapy. Results of the venous blood gas analyses were not so conclusive, but it is influenced also by other factors, such as tissue perfusion and oxygen demand, that’s why it cannot be regarded as a standard method for monitoring the respiratory function. Conclusions In our case the serial arterial blood gas analyses provided adequate information about the ongoing pulmonary inflammatory process and its effect on ventilation.
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DOG’S LUNGWORM DISEASE IN THE CZECH REPUBLIC
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Introduction
In effort to assess the occurrence of two metastrongylid nematodes, Crenosoma vulpis and Angiostrongylus vasorum in dogs in the Czech Republic, a study focused on detection and determination of these two species was performed. A. vasorum is a highly pathogenic nematode which can cause various clinical symptoms as coughing, weakness, vomiting, diarrhoea, haemorrhage, neurological symptoms and even death. Data on clinical signs of C. vulpis infection in dogs are rare and usually include respiratory symptoms. The life cycle is indirect, where dogs acquire infection by the ingestion of terrestrial snails and slug gastropod intermediate hosts.

Materials and Methods
Till now, in total 100 samples of dog’s faecal samples were examined using Baermann larvoscopy method and the data about breed, sex, age, clinical status and locality of the dogs were collected. The larvae were morphologically determined and the DNA isolation, PCR of three fragments of 28S rDNA and sequencing were performed.

Results
Out of 100 samples, 6 were found positive by Baermann method and the larvae were morphologically determined as C. vulpis (5 samples, prevalence 5%) and A. vasorum (1 sample, prevalence 1%). The dogs came from different localities throughout the Czech Republic. All positive dogs except one were without clinical signs of the disease. The symptomatic dog positive for C. vulpis was 3 years old Doberman pinscher male and was suffering from coughing for 2 months. Following haematological examination showed eosinophilia. Other positive dogs for C. vulpis were 3 years old Slovak Cuvac female, 1,5 year old mongrel female, 6 years old English Cocker Spaniel male and 6 months old mongrel male. PCR was positive in all larvoscopy positive dogs; data from sequencing will be presented.

Conclusion
The current study reveals for the first time the presence of two metastrongylid nematodes in dogs in the Czech Republic and shows spreading of A. vasorum from the Western European countries to the Central Europe. The discovery of C. vulpis and A. vasorum in dogs in the Czech Republic indicates that these two parasitic species should be considered in the differential diagnosis of cardiopulmonary diseases in dogs.
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