MEETING ABSTRACTS

Fifth European Dirofilaria and Angiostrongylus Days (FiEDAD) 2016

Vienna, Austria. 11–13 July 2016

Published: 11 January 2017

TOPIC 1: Dirofilarioses (Humans, Mosquitoes)

A1

Human dirofilariosis in Europe: basic facts and retrospective review

F Simón1, V Kartashev2,3, J González-Miguel1, A Rivera4, A Diasdado1, PJ Gómez5, R Mochón4, M Siles-Lucas7

1Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca, Salamanca, 37007, Spain; 2Rostov State Medical University, Rostov-na-Donu, 344022, Russia; 3North Caucasus Research Veterinary Institute, Novocherkassk, 346421, Russia; 4Laboratory of Parasitology, IRNASA, CSIC, Salamanca, 37008, Spain

Correspondence: F Simón (fersimon@usal.es)

In Europe domestic and sylvatic canines and felines are the reservoirs of Dirofilaria immitis and D. repens, while different culicid mosquito species act as vectors of these species. Many mosquito species feed indiscriminately on animal reservoirs and man, thus where there is canine dirofilariosis, the risk of zoonotic infections exists. There are three forms of human dirofilariosis: Pulmonary dirofilariosis (PD), usually causing a solitary pulmonary nodule attributed to D. immitis; subcutaneous dirofilariosis (SD) manifesting as subcutaneous nodules located in different parts of the body and ocular dirofilariosis (OD) in which worms cause nodules or remain unencapsulated in the eye area, being the last two variants mainly caused by D. repens. Most of the information on human dirofilariosis is generated by the clinical cases reported and their retrospective review, but there is very scarce other kind of studies. In Europe continues the sharp increase of SD/OD cases unlike the extremely low number of reports of PD cases, without being able to indicate the objective causes of this fact, since both species are present in animal reservoirs of the continent. Most of these cases have been reported in Ukraine and the Russian Federation [1], although a significant number were detected in recent years in Belarus, Balkan and central European countries. The increase in case reports revealed new locations and clinical implications, which are forcing to reassess the prognosis and severity of many cases. Molecular techniques established that worms of D. repens with ocular localization are genetically identical to those located in the subcutaneous tissue and the participation of D. immitis in OD in Ukraine, where this species seems to be the causal agent of the ocular variant in the 13.8% of cases. The routine application of non-invasive techniques such as ultrasound and Doppler helps to establish a rapid prognosis and diagnosis, consistent with the non-malignant nature of nodules in both SD and OD. Studies using "in vitro" cultures of vascular endothelial and smooth muscle cells have demonstrated the ability of some Dirofilaria molecules to activate the fibrinolytic system and enhance the generation of plasmin. Plasmin plays a dual role contributing to remove thrombi, but also participating in the stimulation of mechanisms leading to villous endarteritis, such as cell proliferation and migration [2]. Although not specifically focused on human dirofilariosis, these studies can contribute to a deeper understanding of the pathophysiology of human dirofilariosis.

References

1. Kartashev V, Tverdokhlebova T, Korzan A, Vedenkov A, Simón L, González-Miguel J, Mochón R, Siles-Lucas M, Simon F. Human subcutaneous/ocular dirofilariosis in Russian Federation and Belarus, 1997–2013. Internat. J. Infect. Dis., 2015, 33:209–11.
2. González-Miguel J, Siles-Lucas M, Kartashev V, Simón F. Plasmin in parasitic chronic infections: Friend or foe? Trends Parasitol., 2016, 32(4):325–35.

A2

Human dirofilariosis – morbidity, clinical presentation, and diagnosis

Vladimir Kartashev1,3, Nikolay Bastrikov1, Boris Ilyasov2, Alexey Ermakov2, Sergey Kartashov2, Denis Dontsov2, Yuri Ambalov2, Tamara Pavlikovskaya2, Olga Sagach1, Svetlana Nikolaenko6, Nina Chizh3, Alla Korzan4, Alena Salayuova4, Javier González-Miguel5, Rodrigo Mochón4, Mar Siles-Lucas6, Fernando Simon7

1Rostov State Medical University, Rostov-na-Donu, 344022, Russia; 2Rostov Oblast Diagnostic Center, Rostov-na-Donu, 344010, Russia; 3North Caucasus Research Veterinary Institute, Novocherkassk, 346421, Russia; 4Central Sanitary and Epidemiological Station of the Ukrainian Ministry of Health, Kiev, 01001, Ukraine; 5Central Sanitary and Epidemiological Station of the Belorusussian Ministry of Health, Minsk, 220000, Belarus; 6Laboratory of Parasitology and IBSAL, University of Salamanca, Salamanca, 37007, Spain; 7Instituto de Recursos Naturales y Agrobiología de Salamanca, CSIC, Salamanca, 37008, Spain

Correspondence: Vladimir Kartashev (vkt@yandex.ru)

As many as 3,545 cases of human dirofilariosis were recognized in Russia, Ukraine, and Belorusussia starting from 1997. Clinical problems of human dirofilariosis become an issue and need be thoroughly analyzed. A patient self-assessment, the parasite anatomical location and clinical manifestation determine diagnostic workup. Five patients with peritonitis were operated immediately and Dirofilaria was unexpectedly found in peritoneal cavity. In contrast – five patients with “silent” pulmonary dirofilariosis were diagnosed late and accidentally. Affected eye (37% of all cases, variations 22 – 48% in different years) in the case of a foreign “moving entity” in an eye or eyelid conjunctiva (19%) or with eye acute inflammation (25%) strongly motivated a patient to visit a doctor in the contrast with patients with slowly growing “silent” nodule (56%). Anyway as many as 86% of the patients with eye located Dirofilaria were addressed to a doctor during the first month of the disease. Nearly equal proportion of patients (around 62%) with head (28%), or trunk (12%), or man’s...
A few thoughts about the recent epidemiological situation of dirofilariosis in Hungary with particular regard to quick spread and high prevalences in certain areas

Eva Fok1, István Kucsera2

1Department of Parasitology and Zoology, University of Veterinary Science, István utca 2., H-1078 Budapest, Hungary; 2Department of Parasitology, National Center for Epidemiology, Albert Flórián út 2-6., H-1097 Budapest, Hungary

Correspondence: Éva Fok (hanavica.fok13@gmail.com)

Parasites & Vectors 2016, 10(Suppl 1):A3

Dirofilaria is an emerging zoonosis in Hungary. The first autochthonous Dirofilaria repens infection of dogs was diagnosed in the end of the 90’s, then soon in 2007 the first dog infected with D. immitis was detected and in 2010 a pet ferret case was published, too. A first comprehensive countrywide survey showed that most of D. repens infected dog cases (prevalence: 18–46%) occurred in the eastern part of Hungary, namely on the Great Hungarian Plain along the Tisza river and its branches [1]. The findings of this earlier study were partly confirmed by later surveys [2, 3, 5], but these studies mainly focused on the heartworm incidence. It is stated [5] that the climate of the Great Hungarian Plain is the most suitable region for the establishment of D. immitis in Hungary. Although sporadic cases in wild canines (such as foxes and jackals) and domestic dogs also occur in other regions of the country it is slightly worrying that the main habitat of D. immitis might be in Szeged town or in the Southern Great Hungarian Plain. This assumption may strengthen by earlier (unpublished) and newer necropsy records [5]. Moreover the first molecular screening of the vector mosquitoes collected in Szeged revealed that not only D. immitis was present in the specimens but also DNA of D. repens [4]. So far, in Hungary human dirofilariosis is caused by D. repens. Since the first reported human case, 115 further episodes were diagnosed in Hungary [6]. Evaluation of the territorial distribution of human episodes revealed that most infections occurred in patients living in the Danube-Tisza interflow region and eastern part of the country. The spread of the “greenhouse effect” lead to the extension of the Mediterranean climatic belt to the north giving better opportunites for both vectors and worms to thrive and spawn infection. A close cooperation not only with the parasitologists, but also between practicing veterinarians and medical doctors is necessary to organise the control against both Dirofilaria species.

References
1. Jacobi O. Prevalence of Dirofilaria spp. in Hungary and veterinary importance, the experience of treatment. PhD thesis, 2014. http://www.hvuteta.hu/bitstream/ handle/10852/1024/Jacobi%20Olga%20Thesis%20English.pdf?sequence=2
2. Farkas R, Gyurovskzy M, Lukács Z, Aladics B, Solymosi N. Seroprevalence of some vector-borne infections of dogs in Hungary. Vector Borne Zoonotic Dis, 2014; 14(4):256–260.
3. Tolnai Z, Szel L, Sproch Á, Szeredi L, Sréter T. Dirofilaria immitis: An emerging parasite in dogs, red foxes and golden jackals in Hungary. Vet Parasitol, 2014; 203 (3-4):339–342.
4. Zittra C, Czuczba Z, Pininyei Sz, Harl J, Kieser K, Laciny A, Barbara Eigner B, Silbermayr K, Duscher G G, Fok É, Fuehrer H-P. Screening blood-fed mosquitoes for the diagnosis of filarioid helminths and avian malaria. Parasites & Vectors, 2015; 8:16.
5. Bacsadi Á, Papp, A, Szeredi L., Tóth, G, Nemes, C, Imre, V, Tolnai, Z, Szel, L., Sréter T. Retrospective study on the distribution of Dirofilaria immitis in dogs in Hungary. Vet Parasitol, 2016; 220:83–86.
6. Dóczi I, Bereczki L., Gyertai T., Fejes I., Skilbek Á., Barcszé Á, Berkes Sz, Tisdavicz L, Barth N, Bende B, Kris E, Kucsera I. Description of five dirofilariosis cases in South Hungary and review epidemiology of this disease for the country. Wien. Klin. Wochenschr, 2015; 127:696–702.
Heartworm disease caused by *Dirofilaria immitis* is well known in Southern parts of Europe. In the past decade several studies have been performed on its diagnosis, treatment and prevention, but knowledge on vector competence of Central European mosquito species for *D. immitis* under local climate conditions is still scarce.

The aim of this study was therefore to analyze the vector competence of three different mosquito species (*Aedes vexans, Ae. geniculatus* and *Culex pipiens*) endemic in Serbia and Switzerland for *D. immitis* at constant and realistic fluctuating temperature regimes under laboratory conditions.

Mosquitoes were kept in the laboratory at 24-27 °C, with 85% of humidity and fed with sugar solution. Six groups of female mosquitoes (30-52 individuals), were fed with blood containing *D. immitis* microfilariae (6,000/ml) obtained from a naturally infected dog using artificial feeding methods (Hemotek, a blood sausage, cotton stick method). After inoculation, blood fed mosquitoes were incubated at constant or realistic fluctuating temperature of 27 °C (17.5-35 °C, with average 27 °C). Parameters determined were: feeding rate, infectious dose, mortality rate, infection rates and infectivity rates. Mosquitoes were dissected under a stereomicroscope on days 2, 4, 7, 10, 14 post inoculation (p.i.), to observe the developmental stages of the filariae. Additionally, PCR analysis was performed. The feeding rate ranged from 26% in *Cx. pipiens* biotype molestus to 61-63% in *Ae. vexans* groups and to 74% in *Ae. geniculatus*. The observed infectious dose was 7-10 microfilaria in *Ae. vexans*. No live microfilariae could be observed in the other species in samples taken on day 1 p.i. Mortality rate was rather high, it was altogether, 78% during the 14 days of incubation period and ranged from 12.5% in *Cx. pipiens* biotype molestus fed with cotton stick to 100% in those fed with Hemotek. The highest percentage (73.9%) of mosquitoes died until day 4 p.i. (feeding). In *Ae. geniculatus* and *Ae. vexans* groups, microfilariae were found until day 7 p.i, L1 from day 4-10p.i. and L2 were found only in *Ae. vexans* groups on day 10. In *Cx. pipiens* biotype molestus no larval stages were found by microscopy. PCR analysis revealed the highest number of specimens positive for *Dirofilaria* in *Ae. vexans*, kept at constant 27 °C. All of the *Cx. pipiens* biotype molestus mosquitoes that died during the experiment were PCR positive. Female mosquitoes developing infectious L3 stages (infectivity rates), L3 were identified only in *Ae. vexans* at day 14 p.i. Total infection rates (microscopy and PCR) were 72% for *Ae. vexans* kept at constant 27 °C, 56.6% for *Ae. vexans* kept at 27 °C fluctuating, 70% for *Ae. geniculatus* and 37.5% for *Cx. pipiens* biotype molestus fed with the Hemotek. To conclude, vector competence for *D. immitis* was shown for the flood water mosquito *Ae. vexans* both at constant and fluctuating 27 °C. The respective results were not yet conclusive for *Cxs pipiens* biotype molestus and *Ae. geniculatus* and further studies will be necessary with these species.
Cortisol, a steroid produced in the adrenal cortex, is a key hormone involved in the stress response and serum levels have often been used as a measure of stress. It has been demonstrated that prolonged stress, as indicated by cortisol levels, is associated with reduced survival, fecundity, and immunity [1]. Studies have examined interactions between parasites and cortisol levels in some species, with discrepant results [2]. The aims of this study were to evaluate the potentially stressful effects of the infection of *Dirofilaria immitis* in dogs by measuring the levels of serum cortisol before and after the adulticide treatment.

Serums from 61 dogs positive to *D. immitis* antigens were included; all blood samples were further examined by the modified Knott test. The parasite burden was assessed by echocardiography in 51 of these dogs [3]. Furthermore, 22 dogs were treated following the AHS protocol and additional blood samples were taken on days 60, 90 and 120. Serum cortisol was measured by EIA Method, validated for this species. There were 24 females and 37 males. Thirty were client-owned dogs and 31 lived in a local shelter; 41 were microfilaremic; 26 were symptomatic and 35 were asymptomatic. When the parasite burden was assessed (n = 51), 20 had high burden and 31 had low burden. The mean level of cortisol in heartworm infected dogs was 10.08 ± 8.16 ng/mL. There were not statistically significant differences between sex and microfilaremic status, but there were between symptomatic and asymptomatic dogs (p < 0.05). When parasite burden was evaluated, dogs with high burden had significantly greater levels of cortisol (p < 0.001). During the adulticide treatment, the levels of cortisol dropped gradually in each sampling, being the cortisol levels from day 120 within the reference ranges (2.31 ± 1.02 ng/mL). Shelter versus client-owned dogs had higher cortisol levels (p < 0.05).

The results demonstrate presence of stress in dogs infected by *D. immitis*, especially in symptomatic dogs, and those with high parasite burden similar to a previous study [4]. These results are similar to other studies which evaluated the effect of several parasites in animals and humans; while the different results found in other research may be caused by the small virulence of the parasites studied [2]. On the other hand, as the parasites are being removed, the levels of cortisol gradually decreased. Although not the aim of the study, we could observe that dogs from shelter had higher levels of cortisol, consistent with previous studies [5].

**Trial registration/ Consent to publish**

The study was approved by the ethical committee of Veterinary Medicine Service of Las Palmas de Gran Canaria University (MV-2016/07) and was carried out in accordance with the current European legislation on animal protection.

**References**

1. Snaith TV, Chapman CA, Rothman JM, Wasserman MD. Bigger groups have fewer parasites and similar cortisol levels: a multi-group analysis in red colobus monkeys. Am J Primatol. 2008; 70: 1072–1080.

2. Monello RJ, Millsbaugh JJ, Woods R, Gompper ME. The influence of parasites on faecal glucocorticoid metabolite levels in raccoons: an experimental assessment in a natural setting. J. Zool. 2010; 282: 100–108.

3. Vénco, L, Gencz, C, Vigevan Colson, P, Kramer, L. 2003. Relative utility of echocardiography, radiography, serologic testing and microfilariae counts to predict adult worm burden in dogs naturally infected with heartworms. In: Seward, R.L., Knight, D.H. (Eds.), Recent Advances in Heartworm Disease, Symposium’01. American Heartworm Society, Batavia, IL, pp. 111–124.

4. Fleming MW. Cortisol as an Indicator of Severity of Parasitic Infections of *Haemonchus contortus* in Lambs (Ovis aries). Comp Biochem Physiol B Biochem Mol Biol. 1997; 116: 41–44.

5. Coppola CL, Grandin T, Enns RM. Human interaction and cortisol: can human contact reduce stress for shelter dogs? Physiol Behav. 2006; 87:537–541.

**Correspondence:** Elena Carretón (elena.carreton@ulpgc.es)

Parasites & Vectors 2016, 10(Suppl 1):A7

**A8 Mitochondrial genome sequences of the zoonotic canine filariae *Dirofilaria (Noctiella) repens* and * Candidatus Dirofilaria (Noctiella) honkongensis***

Esa Yilmaz1, Moritz Fritzenwanker2, Nikola Panchey3, Mathias Lendner4, Sinichit Wongkamch1, Domenico Otranto5, Inge Kroid6, Martin Demenebaum7, Sabrina Ramünke1, Roland Schaper7, Georg von Samson-Himmelstjerna7, Sven Popper1, Jürgen Krücken1, Nikola Pantchev8, Domenico Otranto9, Sabrina Ramünke1, Roland Schaper7, Georg von Samson-Himmelstjerna7, Sven Popper1, Jürgen Krücken1, Nikola Pantchev8, Domenico Otranto9, Sabrina Ramünke1, Roland Schaper7, Georg von Samson-Himmelstjerna7, Sven Popper1, Jürgen Krücken1, Nikola Pantchev8, Domenico Otranto9, Sabrina Ramünke1, Roland Schaper7, Georg von Samson-Himmelstjerna7, Sven Popper1, Jürgen Krücken1, Nikola Pantchev8

1 Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; 2Institute of Medical Microbiology, Justus-Liebig-University, Giessen, Germany; 3German Center for Infection Research (DZIF), Partner site Giessen-Marburg-Langen, Campus Giessen, Giessen, Germany; 4IDEXX Laboratories, Ludwigshurg, Germany; 5Institut für Parasitologie, Universität Leipzig, Leipzig, Germany; 6Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; 7Department of Veterinary Medicine, University of Bari, 70010, Bari, Italy; 8Division of Infectious Diseases and Tropical Medicine, Medical Centre of the University of Munich (LMU), Munich Center for Infection Research (DZIF), Partner site Munich, Munich, Germany; 9Section Clinical Tropical Medicine, Department of Infectious Diseases, Heidelberg University Hospital, Heidelberg, Germany; 10Bayer Animal Health GmbH, 40789, Monheim, Germany; 11University Medical Center, Hamburg-Eppendorf, Germany

**Correspondence:** Esa Yilmaz

Parasites & Vectors 2016, 10(Suppl 1):A8

The vector-borne zoonotic parasite *Dirofilaria repens* causes cutaneous dirofilariosis. In humans, it can manifest as skin nodules in several anatomical regions or subconjunctival infections. Present in the Mediterranean area, many parts of Asia and presumably also in Africa, *D. repens* is apparently expanding its distribution to previously non-endemic areas in the old world. In Hong Kong, a new species, *Candidatus Dirofilaria honkongensis*, has been reported to cause cutaneous and subconjunctival infections of humans. The objectives of this study were to compare mitochondrial genomes from these parasites and to obtain data suitable for population genetic studies. Complete mitochondrial genomes of four adult worms from Italy, Croatia and India were obtained by either PCR followed by Sanger sequencing or Illumina MiSeq. According to cytochrome oxidase I sequences, worms from Europe and India were identified as *D. repens* and C. D. hongkongensis, respectively. This is the first report of C. D. hongkongensis from the Indian subcontinent. The mitochondrial genomes of *D. repens* and *C. D. hongkongensis* are essentially organized like those of other spirurida encoding 2 rRNAs and 12 proteins but lacking the atp8 gene present in most animal mitochondrial genomes. An approximately 2.5 kb fragment was amplified from *Dirofilaria* positive canine blood samples or macrofilaria from Europe (n = 42), Thailand (n = 2) and Vietnam (n = 1) and analyzed together and were most closely related to *Dirofilaria immitis*.
In conclusion, differences between *Dirofilaria* spp. were considerably high while *D. repens* was shown to be genetically quite homogenous. Analysis of mitochondrial sequences supports the hypothesis that C. D. hongkongensis represents a distinct species and suggests that samples from Thailand might represent another cryptic species or a genetically diverged C. D. hongkongensis population. Investigations on a larger geographic scale including representative numbers of samples from regions not analyzed so far as well as development of microsatellite markers for fine mapping would increase our understanding of the population genetics of *D. repens*.

**TOPIC 3: Dirofilarioses (Veterinary Medicine)**

**A9**

Use of histochemical analysis for updates about canine filarioids upon new cases in two dog shelters in the surrounding of Bucharest, Romania

Cristian-Ionut CN Florea1,2, Poliana GH Tudor1, Stefan P Olaru2, Anca M Dobrica2

1Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agronomical Sciences and Veterinary Medicine, Bucharest, 050097, Romania; 2Praxis Vetlife, Bucharest, Romania, 021374

**Correspondence:** Cristian-Ionut CN Florea (florea_christian@yahoo.com)

Parasites & Vectors 2016, 10(Suppl 1):A9

Dogs represent the main natural reservoir for numerous helminths, including some species of filaria that have microfilariae circulating in the blood flow. Of these, the most known canine filarioid species are *Dirofilaria immitis*, *D. repens*, *Acanthocheilonema reconditum* and *A. drancunculoides* transmitted by different vectors (i.e. mosquitoes, fleas, lice and ticks) [1]. In Romania, stray dogs still remain an unsolved issue, despite the efforts made by the authorities in the attempt to gather and place them in shelters towards adoption. The aim of this study is the screening of the infestation with filarioids in new cases brought in two enroled dog shelters surrounding Bucharest, Romania. During November 2014 and October 2015, 282 stray dogs have been brought in two shelters near Bucharest and have been tested. Morphometric analysis of microfilariae canine blood from the enrolled dogs were made by Knott’s modified test. For the detection of the *D. immitis* antigens it was used an in-clinic rapid assay test based on enzyme immunoassay technique (SNAP® 4Dx®, IDEXX Laboratories, Inc., Westbrook, ME, USA). The identification of the species was made by a histochemical technique to demonstrate acid phosphatase activity patterns in the microfilariae, using a comercial kit (Leucognost SP®, Merck, Darmstadt, Germany) in accordance with the manufacturer’s instructions. Out of 282 enrolled dogs, 32.62% (n = 92) were positive for at least one filarial species. The modified Knott’s test showed the presence of circulating microfilariae in 78 samples (27.66%), and the serum of 67 samples was positive for *D. immitis* antigens using an immunoenzymatic assay. As a result of the histochemical technique there were identified three species of microfilariae and the global prevalence was 23.76% (n = 67) *D. immitis*, 9.57% (n = 27) *D. repens* and 0.71% (n = 2) *Acanthocheilonema spp.* In addition, coinfection with *D. immitis* and *D. repens* was found in four samples. Morphometric evaluation showed the following measurements of the length and width of microfilariae: 298.27/5.9 μm of *D. immitis*, 358.81/7.2 μm of *D. repens* and 264/4.6 μm of *Acanthocheilonema spp.* The results of this study highlight the presence of three species of filarioids from the dogs brought to the enrolled shelters with a high prevalence of *D. immitis* (23.75%). This raises public health concern that merits more consideration by both veterinarians and physicians.

**References**

1. Magnis J, Lorentz S, Guardone L, Grimm F, Magi M, Naucke T, Deplazes P. - Morphometric analyses of canine blood microfilariae isolated by the Knott’s test enables *Dirofilaria immitis* and *D. repens* species-specific and *Acanthocheilonema* (syn. *Dipetalonema*) genus-specific diagnosis. Parasit Vectors. 2013; 6:48.

**A10**

Investigations on *Dirofilaria repens* infection in Polish dogs – looking for the objective features of the infection

Artur Dobrzyński1, Maciej Klockiewicz2, Magdalenia Wysmolek1, Michał Czpsowicz1, Marta Parzeniecka-Jaworska1, Joanna Nowakowska2, Ewa Długosz2

1Division of Parasitology and Invasiology, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Ciszewskiego St. 8, 02-786 Warsaw, Poland; 2Department of Small Animal Diseases with Clinic, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Ciszewskiego St. 8, 02-786 Warsaw, Poland; 3Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Ciszewskiego St. 8, 02-786 Warsaw, Poland; 4Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Ciszewskiego St. 8, 02-786 Warsaw, Poland; 5Bayer Animal Health,Aleje Jeronimskie 158, Warsaw, Poland

**Correspondence:** Maciej Klockiewicz (maciej_klockiewicz@sggw.pl)

Parasites & Vectors 2016, 10(Suppl 1):A10

The skin dirofilariosis caused by *D. repens* has been recognized as an emerging disease in Polish dogs. Since first cases were diagnosed almost 10 years ago, now the infection is considered as an increasing epidemiological problem. The extensity of infection in some areas in Poland was estimated over 12-36% [1,2] within the local dogs’ populations. Veterinarians have found this infection as a real threat, so general aim of this research was to find objective features of the infection which could be used to establish the treatment algorithm for vets. The investigation was based on cases reported to Small Animal Clinic of the Warsaw Faculty of Veterinary Medicine as well as of those admitted to other veterinary clinics of Warsaw area. There were 428 dogs preselected (suspected for dirofilariosis) included to this research. Animals underwent physical examinations and blood tests (morphology and biochemistry). At the end of the study results of this preselected group were compared with results obtained from finally diagnosed – infected dogs. Microfilariae were found in 42.8% of examined dogs. Subsequently, PCR and ELISA tests were performed to confirm the infection in possibly infected ones. PCR with differential primers was performed to reveal parasite DNA in blood [3]. ELISA tests were based on adult *D. repens* somatic antigens to detect specific IgG in infected dogs. PCR revealed the additional 8.8% infected dogs. PCR tests also confirmed that all individuals were infected with *D. repens*. Results of ELISA indicate that *D. repens* infection results in high specific IgG titers in more than 80% of infected dogs. ELISA allowed to diagnose over 1/3 additional infected individuals, which have been previously found negative (by blood smear). The blood morphology and biochemistry revealed statistically significant erythrocytopenia, lymphocytopenia, thrombocytopenia, reduced haematocrit, and increased levels of alkaline phosphatase and creatinine in infected dogs. Results suggest that infection is associated with general symptoms and problems of liver and kidneys. Additionally, the comparison between infected and not-infected groups showed that skin dirofilariosis was more often (2.6x) found in dogs which did not received any anti ecto-parasite treatment. The results were used to set up the treatment algorithm for practitioners who are not familiar with this newly emerged disease. It is allowed to suspect infection when similar blood results are obtained, and
Heartworm (HW) infection of dogs is highly prevalent in some areas of Greece [1], but information about the prevention and treatment strategies implemented in the clinical setting is limited. In order to evaluate the perception of veterinarians on the prevalence and their experience on diagnosis, treatment and prevention of HW, a questionnaire survey was designed. The questionnaire was distributed by e-mail to the veterinary practitioners registered to the two major Hellenic veterinary scientific societies. Twenty questions were included, investigating the frequency of HW in each practice and the strategies implemented in the clinical setting is limited. In order to determine the frequency of HW in each practice and the strategies implemented in the clinical setting, a questionnaire survey was conducted. The questionnaire was distributed by e-mail to the veterinary practitioners registered to the two major Hellenic veterinary scientific societies. Twenty questions were included, investigating the frequency of HW in each practice and the strategies implemented in the clinical setting is limited. In order to evaluate the perception of veterinarians on the prevalence and their experience on diagnosis, treatment and prevention of HW, a questionnaire survey was designed. The questionnaire was distributed by e-mail to the veterinary practitioners registered to the two major Hellenic veterinary scientific societies. Twenty questions were included, investigating the frequency of HW in each practice and the strategies implemented in the clinical setting.

A11

Awareness and strategies about canine heartworm (Dirofilaria immitis) infection in private practices in Greece: preliminary results of an ongoing questionnaire survey

Anastasia Diakou1, Mathios Mylonakis2, Zoe Polizopoulou3, Christos Koutinas4

1Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece; 2Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 546 27, Greece; 3Diagnostic Laboratory, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 546 27, Greece

Correspondence: Anastasia Diakou (diakou@vet.auth.gr)

Parasites & Vectors 2016, 10(Suppl 1):A11

Subcutaneous dirofilariosis is a well-known disease caused mainly by Dirofilaria repens and described in several mammalian species including human, dog and cat [1]. Additionally, early developing stages of the heartworm, Dirofilaria immitis, are rarely reported in subcutaneous localization from humans and dogs. To our knowledge, evidence of this condition has not been described in the cat yet, even if the feline host can be affected either by the classic adult-related heartworm form or heartworm-associated respiratory disease (HARD) caused by immature stages. A 2 year-old, spayed male cat was presented for three subcutaneous nodules on the head and trunk. The cat lived in Northern Italy and was regularly vaccinated and treated monthly with an antiparasitic spot on formulation containing selamectin (Stronghold®, Pfizer). One of the three nodules was surgically excised and examined. Histology showed in the subcutis the presence of a nodular lesion characterized by a severe inflammatory infiltrate composed by macrophages, small lymphocytes, with fewer eosinophils and mast cells, supported by a proliferation of mature fibroblasts (fibrosis). Inflammatory cells were multifocally surrounding sections of parasites identified as nematodes. The parasites were characterized by a thick cuticle with a smooth external surface, prominent and large lateral chords and a polynematoid-coelomyarian musculature. Microscopic features were compatible with Dirofilaria immitis DNA. The parasite was amplified with a PCR (ribosomal 5.8S-ITS2-28S region), the PCR product were purified, cloned and thereafter sequenced. A BLAST search revealed 97% identity to D. immitis isolate EU182331 and only 79% of identity the next related sequence of Dirofilaria genus (D. repens). The cat tested negative for D. immitis antigenemia and the two remaining nodules disappeared spontaneously in a few months. Identification of a filaroid nematode with smooth cuticles in the subcutaneous tissues can be challenging. All species of the genus Dirofilaria are characterized by cuticular ridges, except from D. immitis and D. luteae [2], with the latter described so far only in USA in the North American river otter. The parasite in the present case most likely represents an immature stage of D. immitis which developed in the subcutis (L3-L4) and was successively entrapped in this localization. The immunity of the cat, which is not a suitable definitive host for Dirofilaria species, was not clinically significant. One of the nodules was surgically excised and examined. Histology showed in the subcutis the presence of a nodular lesion characterized by a severe inflammatory infiltrate composed by macrophages, small lymphocytes, with fewer eosinophils and mast cells, supported by a proliferation of mature fibroblasts (fibrosis). Inflammatory cells were multifocally surrounding sections of parasites identified as nematodes. The parasites were characterized by a thick cuticle with a smooth external surface, prominent and large lateral chords and a polynematoid-coelomyarian musculature. Microscopic features were compatible with Dirofilaria immitis DNA. The parasite was amplified with a PCR (ribosomal 5.8S-ITS2-28S region), the PCR product were purified, cloned and thereafter sequenced. A BLAST search revealed 97% identity to D. immitis isolate EU182331 and only 79% of identity the next related sequence of Dirofilaria genus (D. repens). The cat tested negative for D. immitis antigenemia and the two remaining nodules disappeared spontaneously in a few months. Identification of a filaroid nematode with smooth cuticles in the subcutaneous tissues can be challenging. All species of the genus Dirofilaria are characterized by cuticular ridges, except from D. immitis and D. luteae [2], with the latter described so far only in USA in the North American river otter. The parasite in the present case most likely represents an immature stage of D. immitis which developed in the subcutis (L3-L4) and was successively entrapped in this localization. The immunity of the cat, which is not a suitable definitive host for D. immitis, likely played a role in preventing migration of the immature stage to the pulmonary arteries. To author’s knowledge this is the first reported case of subcutaneous localization of D. immitis in a feline host.

References

1. Manzocchi S, Lendner M, Pisceddu E, Morabito S, Sebastiani V, Daughthies A, Pantchev N. Nodular presentation of dirofilaria repens infection in a cat mimicking a fibrosarcoma. Vet Clin Pathol. 2015. In press.

2. Orihel CT and Eberhard ML. Zoonotic Filariasis. Clin Microbiol Rev., 1998, 11: 366–381.
A13

In field retrospective study of „slow-kill” treatment efficiency on heartworm positive dogs in general practises in Serbia 
Nenad Milojković1, Momičo Arandželović1, Ljubomir Ćurčin2

1Veterinary Clinic “Vet Centar”, 11000 Belgrade, Serbia; 2Veterinary Clinic “Vet Alfa”, 11000 Belgrade, Serbia; 3Veterinary Clinic “Intervet”, 11000 Belgrade, Serbia. 
Correspondence: Nenad Milojković (nenad.milojkovic66@gmail.com) Parasites & Vectors 2016, 10(Suppl 1):A13

Northern parts of Serbia are hyperendemic for Dirofilaria immitis. A lot of suburban and rural areas may have overall prevalences up to 50% in dogs, and treating them is of great concern for veterinarians in the field. Two limitations make adulticide treatment almost impossible: 1. Imiticide* is not available on the Serbian market and ordering it abroad is expensive for the majority of owners whose dogs are confirmed as Heartworm (HW) positive; 2. It is very difficult, especially in the countryside to do appropriate diagnostics (echocardiography) in order to estimate prognosis after melorsamine treatment. Therefore, the „slow kill” treatment is the only reliably choice for the majority of veterinarians in the field. We have gathered data about HW positive dogs from eleven general practises. The total number of antigen positive dogs was 258. Owners of only 105 dogs were interested to treat their pets. Seven of them have stopped visiting their veterinarians after one month, and 32 dogs with severe disease (respiratory distress, right sided heart failure, caval syndrome) died within the first 3 months after diagnostics. A total number of 66 dogs, with mild and moderate disease, have continued with „slow- kill” treatment. Schedule for treatment was intermittent application of doxycyclin (10 mg/kg every third month) and prophylactic dose of ivermectin (10 mcg/kg every 15 days). Therapy was stopped until the first negative antigen test. During the first 3 months, 9 (13.64%) patients became antigen negative, between 3 and 6 months 3 (4.54%), between 6 and 9 months 29 (43.94%) and 14 (21.24%) between 9 and 12 months; 11 (16.64%) dogs become negative after more than 12 months. All dogs from the last group did not visit veterinarians regularly and were not on continuous therapy. We also want to remark that a lot of dogs in the study were not tested each month. Most of them were retested twice annually, due to financial capabilities of their owners. Data from the study shows frequent owners neglecting to do any further diagnostics and treatment of their HW positive dogs in suburban and rural areas. Those dogs persist as a reservoir for the disease. This fact demands more active education of the owners. On the other hand, dogs of committed owners, with mild and moderate disease, which are going to „slow kill” treatment, have a good chance to be cured.

References
1. Grandi G, Quintavalla C, Mavropoulou A, Genchi M, Grundi G, Bertoni G, Kramer L. A combination of doxycycline and ivermectin is adulticidal in dogs with naturally acquired heartworm disease (Dirofilaria immitis).Veterinary Parasitology, Volume 169, Issues 3–4, 11 May 2010, Pages 347-351
2. Mavropoulou A, Grundi G, Grandi G, Volta A, Kramer L, Quintavalla C. Clinical assessment of post-adulticide complications in Dirofilaria immitis-naturally infected dogs treated with doxycycline and ivermectin; Veterinary Parasitology, Volume 205, Issues 1–2, 15 September 2014, Pages 211-215
3. Venco L, McCall J, Guerrero J, Genchi C. Efficacy of long-term medical administration of ivermectin on the progress of naturally acquired heartworm infections in dogs, Veterinary Parasitology, Volume 124, Issues 3–4, 1 October 2004, Pages 259-268
4. McCall J, Genchi C, Kramer L, Guerrero J, Dzimianski M, Supakorndej P, Mansour A, McCall S, Supakorndej N, Grandi G, Carson B. Heartworm and Wombbacha: Therapeutic implications. Veterinary Parasitology, 2008, 158, 204–214.
5. http://www.escap.org Control of vector- borne diseases in dogs and cats; ESCAP Guideline 05 second edition – October 2012. 
6. http://www.heartwormsociety.org Current Canine Guidelines.
7. Rawlings C, Calvert C. Heartworm disease, in Ettinger S, Elsevier Sounders, 1995, (1046-1067)
8. Atkins C, Canine heartworm disease; Feline heartworm disease, in Ettinger S, Elsevier Sounders, 2005, (1118 – 1144).

A14

The true story of Dirofilaria in the Czech Republic
Barbora Mitkova1, 2, Marcela Novotna2, Jana Jurankova3, Lada Hofmannova1, Dwight D. Bowman4, David Modry1, 2, 3

1Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno 61242, Czech Republic; 2CEITEC VUF, University of Veterinary and Pharmaceutical Sciences, Brno 61242, Czech Republic; 3Department of Microbiology & Immunology, College of Veterinary Medicine Cornell University, Ithaca, NY 14853, USA; 4Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Ceske Budelovice 370 05, Czech Republic. 
Correspondence: Barbora Mitkova (bmitkova@pobox.sk) Parasites & Vectors 2016, 10(Suppl 1):A14

The first autochthonous infection of Dirofilaria repens and Dirofilaria immitis in the Czech Republic were reported in 2006 [1] using several diagnostic methods for detections of these parasites. Since then, Dirofilaria infection was repeatedly reported in dogs [2] and, recently, D. repens was detected also in mosquitoes [3] and in humans [4]. The presence of D. immitis in the Czech Republic was established only on detection of antigen using the commercially available test. In past 10 years, detection of D. immitis microfilariae, PCR detection or clinical case of canine dirofilariosis caused by D. immitis were not reported from the Czech Republic. The aim of presented survey was to confirm or exclude the autochthonous infection of D. immitis in dogs from the Czech Republic and to determine the extent of endemic distribution of D. repens within the Czech Republic. A total number of 392 blood samples from dogs were examined using the modified Knott test, IDEXX SNAP® 4Dx® test and PCR amplifying the fragment of COI gene of filarial nematodes [5]. Only 3 cases were detected by Knott test and/or by PCR with prevalence 6.4% (25/392). Six out of 25 positively diagnosed dogs had no travel history outside the Czech Republic, so the autochthonous infection was proven in 3.4% animals. Almost all positive dogs had originated from Southern Moravia region except a single one, which was from Zlin region, 100 km north of other positive localities. D. repens prevalence demonstrated in our sample set is lower than previously published (9–24%), however, distribution of positive animals corresponds well with published presence of D. repens positive mosquitoes and with occurrence of cases of autochthonous human dirofilariosis from the same region. Our study confirmed the endemic occurrence of D. repens in the region of Southern Moravia in the Czech Republic. Importantly, no D. immitis was detected. Based on these results, and considering total absence of published clinical cases, microfilariae or PCR detection of D. immitis in the Czech Republic, we strongly recommend not to consider the Czech Republic as currently endemic for this parasite.

This study was supported by COST CZ LD14048; survey was organized in the framework of the EurNegVec COST Action TD1303.

References
1. Svobodová Z, Svobodová V, Genchi C, Forejt P. The first report of autochthonous dirofilariosis in dogs in the Czech Republic. Helminthologia. 2006; 43:242–245. doi:10.2478/s11687-006-0046-5
2. Dobělová R, Svobodová V. Progressive spread of Dirofilaria infections in dogs along rivers in the southeastern Czech Republic. In: Mochon R, Simón F., Montoya J. A., Genchi C. 2nd European Dirofilaria Days, Programme and abstract SEDD 2009; O 4, p.190.
3. Rudolf I, Sebesta O, MendeI J, Betášková L, Bocková E, Jedličková P, Vencílková K, Blážejová H, Šikutová S, Hubálek Z. Zoonotic Dirofilaria repens (Nematoda: Filarioidea) in Aedes vexans mosquitoes, Czech Republic. Parasitol Res. 2014; 113:4663–4667. doi:10.1007/s00436-014-4191-3
4. Matejů J, Chanová M, Modrý D, Mitkova B, Hrazdilová K, Zampachová V, Kolárová L. Dirofilaria repens: emergence of autochthonous human infections in the Czech Republic (case reports). BMC Infect Dis. 2016; 16:171. doi:10.1186/s12879-016-1505-3
5. Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C, Genchi C. A phylogenetic analysis of filarial nematodes: comparison with the
Canine dirofilariosis has rarely been diagnosed in Austria before 2008. All dogs had a history either originating from an endemic country or staying abroad for a certain time. Dogs were identified by accidental finding of microfilaria in blood or urine samples or by the directed detection of adult *D. immitis* or *D. repens* [1, 2]. From 2008 on case numbers increased rapidly regarding both infections. The typical origin from Mediterranean countries in dogs with heartworm disease has been replaced by the origin from Eastern countries, led by far by cases from Hungary (Fig. 1). Several animal welfare associations located in Austria financially support foreign animal shelters in neighbouring countries and organize dog importation to Austria and Germany on a large scale. Unfortunately, most of these animals are not tested for dirofilariosis prior to importation and they are not protected by microfilaricides to avoid local transmission to mosquitoes. First canine cases of *D. repens* infections with probable autochthonous background have been diagnosed in Austria in 2008 [3]. In 2014 first detection of *D. repens* in vector mosquitoes has been reported [4] and confirmed by additional autochthonous canine cases. An obvious increase of imported dogs from eastern countries to Austria has been recognized within the last five years, concurrently canine heartworm disease cases increased markedly, too (Fig. 2). Several criteria turned out to be important in the consultation talk to the dog’s owner for the decision to have these animals on therapy:

1) Several animal welfare association members refuse heartworm therapy in dogs due to possible side effects. Side effects and lethality rates were massively overstated and erroneously reported to the owners.

2) Estimated costs are high, especially when following the guidelines from the American Heartworm Society.

3) None of the owners was informed about the possible influence of importing infected dogs to Austria regarding endemicity and zoonotic hazards.

To offer a safe and affordable therapeutic regime, a modified scheme has been introduced to these animals including two injections of melarsomine three days apart, and oral medication of macrocyclic lactones to avoid local transmission to mosquitoes. Formulation on the occurrence of *D. repens* in Austria financially support foreign animal shelters in neighbouring countries and organize dog importation to Austria and Germany on a large scale. Unfortunately, most of these animals are not tested for dirofilariosis prior to importation and they are not protected by microfilaricides to avoid local transmission to mosquitoes. First canine cases of *D. repens* infections with probable autochthonous background have been diagnosed in Austria in 2008 [3]. In 2014 first detection of *D. repens* in vector mosquitoes has been reported [4] and confirmed by additional autochthonous canine cases. An obvious increase of imported dogs from eastern countries to Austria has been recognized within the last five years, concurrently canine heartworm disease cases increased markedly, too (Fig. 2). Several criteria turned out to be important in the consultation talk to the dog’s owner for the decision to have these animals on therapy:

1) Several animal welfare association members refuse heartworm therapy in dogs due to possible side effects. Side effects and lethality rates were massively overstated and erroneously reported to the owners.

2) Estimated costs are high, especially when following the guidelines from the American Heartworm Society.

3) None of the owners was informed about the possible influence of importing infected dogs to Austria regarding endemicity and zoonotic hazards.

To offer a safe and affordable therapeutic regime, a modified scheme has been introduced to these animals including two injections of melarsomine three days apart, and oral medication of macrocyclic lactones and doxycyclin. Transmission risk was reduced immediately and 22/26 became negative in the antigen test within 4–8 months after melarsomine injections. Informing and advising animal welfare associations and dog owners as well as identification of imported and infected animals and the rapid onset of a safe therapy is the only way to delay or even avoid heartworm disease becoming endemic in Austria.

References

1. Leschnik M, Löwenstein M, Edelhofer R, Kertz G. Imported non- endemic, arthropod-borne and parasitic infectious diseases in Austrian dogs. Wien Klin Wochenschr. 2008;120(19-20 Suppl 4):S9–S2.

2. Fuehre HP, Auer H, Leschnik M, Silbermayr K, Duscher G, Joachim A. Dirofilaria in Humans, Dogs, and Vectors in Austria (1978-2014)-From Imported Pathogens to the Endemicity of Dirofilaria repens. PLoS Negl Trop Dis. 2016; 10(5):e0004647.

3. Duscher G, Feiler A, Wilé-Pizzai W, Bakonyi T, Leschnik M, Miterpáková M, Kolodziejej J, Nowotny N, Joachim A. Detection of Dirofilaria in Austrian dogs. Berl Munch Tierarztl Wochenschr. 2009; 122(5-6):199-203.

4. Silbermayr K, Eigner B, Joachim A, Duscher GG, Seidel B, Allerberger F, Indra A, Hufnagl P, Fuehrer HP. Autochthonous Dirofilaria repens in Austria. Parasit Vectors. 2014; 7:226.
of *D. immitis* in red foxes from Portugal and to evaluate their poten-
tial role in the epidemiology of dirofilariosis. Blood (n = 94) or meat
juice (n = 25) were obtained from 119 wild red fox carcasses shot
during the official hunting season or killed on the road due to traffic
accidents between 2008 and 2010. These animals came from eight
districts of northern (Braga, Bragança, Porto, Viana do Castelo and
Vila Real), central (Aveiro) and southern Portugal (Évora and Setúbal).

*D. immitis* circulating antigens were detected using a commercially
available enzyme-linked immunosorbent assay (ELISA) antigen kit,
(WITNESS® Dirofilaria; Syntec, Europe). Of the 119 foxes, 10
(8.4%; CI: 4.1-14.9%) were found infected with *D. immitis*, with posi-
tive animals found in five districts (Braga, Bragança, Évora, Viana
du Castelo and Vila Real), in northern and southern areas of Portugal.
One of the samples that were positive to *D. immitis* was obtained
with meat juice, a finding which suggests that it could be used as an
alternative sample to serum for the antigen detection of antigen, in
post-mortem analysis. The present report demonstrates that infection
with *D. immitis* is prevalent in red fox populations in Portugal, show-
ing an increase of prevalence compared with recent reports [4, 5] and
suggesting a role of these animals as potential reservoir hosts for
domestic pets and even to humans. Given the complex interaction
between wildlife and domestic animals, humans and parasites, a robust
health risk surveillance assessment should be implemented in Por-
guese fox population to allow a better management of its vector-
borne infections and diseases, in line with the ‘One Health’ concept.

**Funding**
PhD Research Grant SFRH/BD/85427/2012; Projects UID/CVT/00276/
2013 CiISA-FMV-ULisboa and GHTM-UID/Mult/04413/2013, supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal.

**References**
1. Otranto D, Cantacessi C, Pfeffer M, Dantas-Torres F, Brianti E, Deplazes P,
    Vilhena H, Granada S, Oliveira AC, Schallig HD, Nachum-Biala Y, Cardoso
    L, Baneth G. Serological and molecular survey of *Dirofilaria immitis* infection
    in dogs from Luanda, Angola. Parasit Vectors., 2014, 7: 114.
2. Cardoso L, Oliveira AC, Granada S, Nachum-Biala Y, Gilad M, Lopes AP,
    Sousa SR, Vilhena H, Baneth G. Molecular investigation of tick-borne path-
    ogens in dogs from Luanda, Angola. Parasit Vectors., 2016, 9: 252.
3. Velazquez L, Blagburn BL, Duncan-Decoo R, Johnson EM, Allen KE, Meinkoth
    J, Gruntmeir J, Little SE. Increased prevalence of *Dirofilaria immitis* antigen
    in canine samples after heat treatment. Vet Parasiol, 2014, 206:67–70.
4. Nelson CT, McCall JW, Canthers D. Current canine guidelines for the diagno-
sis, prevention, and management of heartworm (*Dirofilaria immitis*) in-
fection in dogs. American Heartworm Society, 2014; https://
www.heartwormsociety.org/.
5. Bwambaloi O, Frank H. The incidence and pathology of *Dirofilaria immitis*
infection in dogs in Nairobi. J Small Anim Pract. 1970(11(5)):293–300.
6. Schwann EV, Durand DT. Canine filariosis caused by *Dirofilaria immitis* in
    Mozambique: a small survey based on the identification of microfilariae. J
    Vet Parasitol., 2002, 73: 124–126.
7. Siwila J, Mwase ET, Nejsum P, Simonsen PF. Filarial infections in domestic
dogs in Lusaka, Zambia. Vet Parasitol, 2015, 210: 250–254.

**POSTER SESSION**

**A18 A skin nodule due to *Dirofilaria repens* in a Tosa dog in Ile de France**
Radu Blaga1, Virginie Daniel-Lesnard2, Bruno Polack3, Stéphanie Beurelt3,
Coraille Martin3, Jacques Guillot1
1Parasitology department, BioPôle d’Alfort, Ecole nationale vétérinaire
    d’Alfort, UPE, Maisons-Alfort, France; 2Clinique vétérinaire du Chêne
    Rouge, Chanteloup-en-Brie, France; 3Vebio laboratory, Arcueil, France;
4Muséum national d’Histoire naturelle, Paris, France
Correspondence: Jacques Guillot (jacques.guillot@vet-alfort.fr)
Parasites & Vectors 2016, 10(Suppl 1):A18
**References**

1. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carreton E, Montoya-Alonso JA. Human and animal dirofilariosis: the emergence of a zoonotic mosaic. Clin Microbiol Rev. 2012; 25 (3): 507–544.

A20

Molecular investigation of possible *Dirofilaria repens* vertical transmission from queen to offspring - case report from Poland

Ewa Długosz1, Agnieszka Szmidi2, Artur Dobrzyński2, Magdalena Wysmolek1, Maciej Klockiewicz1

1Division of Parasitology and Invasiology, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Ciszewskiego St. 8, 02-786 Warsaw, Poland; 2Out-patients veterinary clinic “Przy Forcie”, Obrorówców Tobruki St. 27 lok. 4, 01-494 Warsaw, Poland; 3Department of Small Animal Diseases with Clinic, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Nowoujowskowsa St. 159, 02-786 Warsaw, Poland

Correspondence: Ewa Długosz (ewa_dlugosz@sggw.pl)

Parasites & Vectors 2016, 10(Suppl 1):A20

A stray queen with her offspring was delivered to the veterinary clinic in Warsaw. The litter consisted of 3 female and 4 male kittens. The age of kittens was estimated around 8 weeks. During physical examination all of them were found in poor condition. The family was severely infested with fleas. Fecal examination results showed that the queen was infected with *Toxocara sp.*, *Ancylostoma sp.*, and *Dipylidium sp.*, and the offspring with roundworms and hookworms. Some of the kittens manifested diarrhea and also conjunctivitis was noticed. A blood sample was collected from the adult cat to check its general status. During the examination some individual microfilariae were found. Blood samples were then taken from three kittens and blood smears revealed the presence of single microfilariae in two of them. Regarding the severity of the circumstances the veterinarian decided to apply moxidectin/imidacloprid topical solution (Advocate®, Bayer) and other necessary treatment. Two days later the veterinarian contacted our laboratory in the Division of Parasitology at the Faculty of Veterinary Medicine. We asked for blood and serum samples which were then taken from the queen and the kittens and delivered to our laboratory. In order to confirm the infection genomic DNA was isolated from blood samples and PCR was performed [1]. PCR product specific for *D. repens* was amplified only in the sample originating from the queen which was taken before treatment. In queen and kitten blood samples which were taken after treatment PCR results were unambiguous. The presence of *D. repens* specific antibodies in all examined sera was confirmed by ELISA. The highest titer was noted in queen serum (1/25600). Titors in kitten sera were lower and ranged from 1/3200 to 1/800. Vertical transmission of filarial infections is uncommon. Only few cases of transplacental transmission of microfilariae have been reported: *Brugia phanangi* in the cat [2], *Dirofilaria immitis* in the dog [3], *Wuchereria bancrofti* and *Onchocerca volvulus* in humans [4, 5]. Our results allow to hypothesize that *D. repens* vertical transmission occurred in investigated cats. At the same time, it is very unlikely that kittens had been infected by another way at this age. In conclusion, there are many questions to be answered. What was the actual route of transmission in this particular case? What is the pattern of immune response against *D. repens* in cats? More research should be conducted in order to provide the adequate control measures to prevent skin dirofilariosis in pets and humans.

**References**

1. Gioia G, Lecová L, Genchi M, Ferri E, Genchi C, Mortarino M. Highly sensitive multiplex PCR for simultaneous detection and discrimination of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood. Vet Parasitol. 2010, 172:160–136.

2. Rimmig P. Diaplacental transmission of microfilaria of the species, *Brugia pahangi*, in the cat. Z Parasitenkd. 1979; 58:181–186.

3. Mantovani A, Jackson RF. Transplacental transmission of microfilariae of *Dirofilaria immitis* in the dog. J Parasitol. 1966; 52:116.
Human dirofilariosis caused by a Dirofilaria repens is relatively rare zoonotic infestation, but according to the literature number of reported cases increase in Serbia, the Balkans and other European countries in the last 10 years [1, 2, 3]. Recently, the parasite was identified by molecular techniques in Culex pipiens and Aedes vexans in Serbia [4]. About 37 cases of superficial (subcutaneous and eye infections) and visceral infections were reported in Serbia till 2015 with predominant subcon- nucital and periorcular infestation. Aim of this paper is to report new D. repens infections diagnosed in our country, and to address attention that this mostly benign infection may have serious clinical course. We present three cases of human dirofilariosis, two autochthonous and one imported, diagnosed from February 2015 till April 2016. All patients were adults, one male and two females, two with subcutaneous infec- tion on the limbs and one with infection of the eye. The male patient, 57 years old is a resident of Belgrade who frequently travelled to Novi Pazar which is located in the southern part of Serbia. He presented with five days history of pain, swelling and redness on the anterior part of the right thigh (6 × 12 cm) near inguinal area, temperature 38 ° C and eosinophilia (10.3). According to the clinical picture and ultrasound findings phlegmona and cellulites were diagnosed and ceftriaxone (2 g IV 5 days) was prescribed. Although pain and redness disappeared, and nodular swelling was clearly defined (5 cm), needle puncture was per- formed and white thread like 8 cm long mass was extracted and amoxicillin/clavulanic acid (100 mg twice/day for 15 days) was pre- scribed. Diagnosis of a D. repens infection was made in pathohistologi- cal preparations and the infectiologist prescribed ivermectin (200mcg/ kg PO once). He has been on follow-up for two months without any signs of recurrence. At the ocular case, a 64 year old woman from Belgrade was presented with history of progressive swelling, redness, pain and unpleasant feeling in the right eye conjunctival area. Under ophthalmic examination mass with thin, very active and movable worm wrapped in circles was found. The 8 cm worm was surgically removed under local anesthesia (Figs. 3 and 4). The third case of Dirofilaria infec- tion was imported from Tivat, Montenegro in a 22 year old female. The infection was manifested as two weeks increasing subcutaneous nod- ule (3.5 cm) of the anterior forearm near elbow crease accompanying with pruritus, erythema and pain (Fig. 5). Routine blood tests, including eosinophil count were within normal limits. Abscess was performed and 9 cm worm was extracted. In the previous two cases D. repens was identified according to mor- phological features in histological sections. All patients provided agreement for participation in this study.

References
1. Džamčić AM, Čolović N, Arsić Arsenijević VS, Stepanović S, Borić I, Džamčić Z, Mitrović SM, Rašić DM, I Stefanović, Latković Z, Kranjčić Zec I. Human Dirofilaria repens infection in Serbia. J Helminthol. 2009; 83:129–137.
2. Tasić-Otašević SA, Trenkić-Džamčić MS, Gabrielli SV, Genchi C. Canine and human Dirofilaria infection in the Balkan Peninsula. Vet Parasitol. 2015; 209:151–156.
Several species of family culicidae mosquitoes have been identified as vectors of canine and feline cardiopulmonary dirofilariosis in different parts of the world. Its transmission depends mainly on weather conditions, which must be favorable for their development and survival. Europe is a continent where heartworm disease is expanding, but, at the same time, there are very few studies about the transmission vectors. Our aim is to review the current distribution of these vectors in the European continent, the changes and their possible causes. Various studies had reported several species of mosquitoes infected by D. immitis larvae such as Haplotype H1 of Culex pipiens in Spain; Cx. pipiens complex in Italy, Turkey, Germany and Belarus Republic; Cx. torrentium in Germany and Belgium; Cx. theileri in Madeira (Portugal) and Canary Islands (Spain); Aedes albopictus, Ae. caspius and Coquillettidia richardi in Italy; Anopheles maculipennis in Italy; Ae. vexans in Turkey, Slovakia and Czech Republic. On the other hand, a few species of mosquitoes have been described as D. repens potential vectors: An. maculipennis and An. algeriensis in Austria; An. daciae, Culiseta annulata and An. maculipennis in Germany; Ae. vexans in Slovakia and Germany and An. claviger s.l. in Belarus. In these studies have been employed or CO2 or animal or human-bait traps [1-7]. Several factors can exert an influence on the emergence or discovery of species or new species to act as vectors, such as the climate change caused by the global warming or the interest in studying this disease in countries when new cases of canine heartworm are diagnosed. The activity of these species is another factor to consider. Mainly, their activity develops in spring and summer and their behavior depends on the different feeding patterns of each species. For example, Cx. pipiens, Anopheles spp. are active only during the night while Ae. Albopictus predominantly at dawn or during the day. More studies and new programs of control of vectors in the current and new endemic countries, and control measures should be carried out to prevent the spreading of this disease.

References
1. Bocková E, Iglodíjová A, Kočičová A. Potential mosquito (Diptera: Culicidae) vector of Dirofilaria repens and Dirofilaria immitis in urban areas of Eastern Slovakia. Parasitol Res. 2015; 114: 4487–4492.
2. Kronefeld M, Werner D, Kampen H. PCR identification and distribution of Anopheles dacieae (Diptera, Culicidae) in Germany. Parasitol Res. 2014; 113: 2079–2086.
3. Montani F, Ciocchetta S, Devine G, Ravagnan S, Mutinelli F, Frangiapani di Regalbino A, Otranto D, Capelli G. Development of Dirofilaria immitis within the mosquito Aedes (F.Control) koreicus, a new invasive species for Europe. Parasit Vectors. 2015; 8: 177.
4. Mochón R, Carretón E, González-Miguel J, Mellado-Hernández I. Heartworm Disease (Dirofilaria immitis) and Their Vectors in Europe - New Distribution Trends. Front Physiol. 2012; 3: 196.
5. Rudolf I, Sebesta O, Mendeš J, Betálková L, Bocková E, Jedličkovičová P, Venclovičková K, Blábová-H. Svitáková S, Hubálek Z. Zoocotic Dirofilaria repens (Nematoda: Filarioidea) in Aedes vexans mosquitoes, Czech Republic. Parasitol Res. 2014; 113: 4663–4667.
6. Sassnau R, Czajka C, Kronefeld M, Werner D, Genchi C, Tannah E, Kampen H. Dirofilaria repens and Dirofilaria immitis DNA findings in mosquitoes in Germany: temperature data allow autochthonous extrinsic development. Parasitol Res. 2014; 113: 3057–3061.
7. Sulejko T, Volkova T, Yashkova S, Tomazatos A, van Thien H, Lütken R, Tannah E. Detection of Dirofilaria repens and Dirofilaria immitis DNA in mosquitoes from Belarus. Parasitol Res. 2016 May 11. [Epub ahead of print]

A22
Heartworm disease vectors in Europe – new distribution trends
Rodrigo Mochón1, Elena Carretón1, Paula Josefina Gómez2, Alicia Díosdado2, Javier González-Miguel1
1Laboratory of Parasitology, University of Salamanca, Salamanca, 37007, Spain; 2Internal Medicine, University of Las Palmas de Gran Canaria, Gran Canaria, 35413, Spain
Correspondence: Rodrigo Mochón (rmorgar@usal.es)
Parasites & Vectors 2016, 10(Suppl 1):A22

A23
Distribution and prevalence of heartworm disease in the canine population in the province of Salamanca (West-central Spain)
Alicia Díosdado, Javier González-Miguel, Fernando Simón, Rodrigo Mochón
Laboratory of Parasitology, University of Salamanca, Salamanca, 37007, Spain
Correspondence: Rodrigo Mochón (rmorgar@usal.es)
Parasites & Vectors 2016, 10(Suppl 1):A23

Since dirofilariosis caused by Dirofilaria immitis is a vector-borne disease, its distribution depends on environmental conditions as well as demographic factors and the management of pets by humans [1]. In the province of Salamanca (West-central Spain) the disease is known from many years, appearing in an area with extensive irrigated crops along the river Tormes [2]. Because recent demographic changes have occurred in this area, the present study has been carried out with the aim to monitoring the distribution and prevalence of the disease in the canine population of this area. For that, 191 dogs were analysed through antigen and microfilariae tests and geo-referenced in a map. The general prevalence is 5.76%, although the disease is only present in dogs from municipalities with irrigated crops in which the prevalence is 16.67%. These results indicate that D. immitis continues to be present in the province of Salamanca and that it is associated with the presence of irrigations but with a clear decrease in the prevalence. Causes of the prevalence decrease as well as the potential zoonotic risk are discussed.

References
1. Simón F, Siles-Lucas M, Mochón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev. 2012; 25: 507–544.
2. Mochón R, Carretón E, González-Miguel J, Mellado-Hernández I. Heartworm Disease (Dirofilaria immitis) and Their Vectors in Europe - New Distribution Trends. Front Physiol. 2012; 3:196.

A24
Diagnostics and therapy of Dirofilaria immitis infections in an isolated dog shelter
Vladan Panic1, Rastko Bekvalac2, Ivan Fenjac3, Aleksandar Potkonjak2, Suzana Otasevic2, Sara Savic3
1Private veterinary practice “Pedigre”, Novi Sad, Serbia; 2Department of Veterinary medicine, Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia; 3Department of Microbiology and Immunology, Medical faculty, University of Nis, Public Health Institute Nis, Nis, Serbia; 4Scientific Veterinary Institute “Novi Sad, Novi Sad, Serbia
Correspondence: Sara Savic (sara@niv.rs.rs)
Parasites & Vectors 2016, 10(Suppl 1):A24
The first published research on *Dirofilaria immitis* (*D. immitis*) infec-
tions in Serbia was in the 1990s, when the first cases were deter-
mimed in dogs, discovered as a side finding during dissections. So-
far, after many studies, it can be pointed out that Vojvodina,
(Northern Serbia) is an endemic region for dirofilariasis in dogs
caused by *D. immitis*. During the period of the last 10 years,
prevalence of *D. immitis* infection in dogs went from 7% to
26.9%. Today, clinical symptoms in dogs can be observed, a regu-
lar health check-up in dogs is provided by the veterinary service.
Herein we report a very high prevalence of *D. immitis* infec-
tions in dogs from one dog shelter with a total of 19 dogs near Novi
Sad, Vojvodina and good outcomes after Ivermectin therapy. The
shelter is situated 20 km away from the city of Novi Sad, close
to the river Danube, with a lot of trees and grass surfaces
around. Out of a total of 19 dogs, 13 dogs had *D. immitis* infec-
tions which were diagnosed at clinical examinations (dogs presented
cough and weakness during the regular everyday activities), snap
test (SNAP 4DX* index) and by Knott test for detection of microfili-
ae in peripheral blood. In all infected dogs, therapy was started
with Ivermectin, with a dose of 0.6 mg/kg per body mass every
week for 4 weeks, then every two weeks, followed by once per
month. The monitoring of therapy effectiveness was performed
every month in all of the dogs due clinical examination and
Knot test. After therapeutic procedure, microfilariae were not
 detected in blood of all cured dogs. Therapy with Ivermectin and
Knot test were repeated for the next 6 months and there were
no parasitological positive findings of dirofilariasis. In addition,
eight months after the therapy was given to all of the dogs, parasit-
alotelogical and clinical examinations showed that all dogs
were without clinical symptoms and using Knot test microfilariae
were not found in blood of examined dogs.

A25

Detection of *Dirofilaria* spp. in dogs from Greece: Preliminary
results

Elias Papadopoulos1, Athanasios Angelou1, Eletherios Gallidis1,
Kyriakos Spanoudis1, Roland Schaper2, Ramsawamy Chandrashekhar3
1School of Veterinary Medicine, Aristotle University, Thessaloniki, 541 24, 
Greece; 2Bayer Animal Health GmbH, Leverkusen, 51368, Germany;
3IDEXX Laboratories, Inc., Westbrook, Maine 04092, USA

Correspondence: Elias Papadopoulos (eliaspap@vet.auth.gr)

Parasites & Vectors 2016, 10(Suppl 1):A25

Dirofilaria is an important parasitic disease of dogs, cats and
wild carnivores worldwide. It is among the most common canine
vector-borne disease and represents a serious threat. It provokes a
parasitological and clinical changes in dogs, discovered as a side finding during dissections. The results revealed a high occurrence of *Dirofilaria* spp. in clinically healthy dogs in Greece and highlight the need to maintain a comprehensive and regular prophylaxis to reduce the contact between dogs and mosquito vectors. Furthermore, the findings of this study confirm that clinically healthy dogs need to be routinely screened for this parasite, as early diagnosis may be an important component of successful treatment and public health protection.

The study was funded by Bayer Animal Health GmbH.

References

1. Milanis D, Meditiskou S, Kelekis A, Papachristos I. Human pulmonary
Dirofilaria: one more case in Greece suggests that *Dirofilaria* is a rather
common cause of coin lesions in the lungs in endemic areas of Europe.
Int J Immunopathol Pharmacol. 2010, 23: 345–348.
2. Polizopoulou ZS, Koutinas AF, Saridomichelakis MN, Patskas MN, 
Leontids LS, Roubies NA, Desiris AK. Clinical and laboratory observations
in 91 dogs infected with *Dirofilaria immitis* in northern Greece. Vet Rec. 
2000; 146: 466–469.

A26

Subjective and objective assessment of radiographic findings in
dogs with heartworm disease

Ljubica Spasojevic Kosic1, Vesna Lalosevic1, Aleksandar Naglic2, 
Stanislav Simin1, Ljiljana Kuruca1, Aleksandar Spasovic1
1Department of Veterinary Medicine, Faculty of Agriculture, University of
Novi Sad, Novi Sad, 21000, Serbia; 2JKP ‘Zoohigijena i veterina’, Novi
Sad, 21000, Serbia; 3PVA ‘Mama’, Belgrade, 11000, Serbia

Correspondence: Ljubica Spasojevic Kosic (ljubicaskosp@gmail.com)

Parasites & Vectors 2016, 10(Suppl 1):A26

Thoracic radiography is a very important diagnostic procedure for
establishing a diagnosis of the heartworm disease (HWD). It en-
ables an insight into the morphology of a lung field and cardiac
silhouette. Radiographic changes associated with HWD can be
assessed both subjectively and objectively. The aim of this work
is to score subjective changes associated with canine heartworm
disease in order to make them more comparable and useful for
clinicians. Within objective measurements, in addition to the de-
termination of a heart size, sizes of relevant blood vessels were
determined according to the vertebral heart scale (VHS) system.
Thoracic radiographs from 20 dogs with natural heartworm dis-
 ease were measured. Both recumbent lateral (LL) and dorsoven-
tral (DV) radiographs were available from 16 dogs whilst lateral
recumbent radiographs were available from 4 dogs. The diagnosis
of the heartworm infestation was established according to the re-
sults of wet blood smears, modified Knot test [1] and heartworm
antigen test. Radiographs of each dog were assessed subjectively
(vascular and cardiac changes) and objectively (VHS) [2] and
subjectively (VHS) [3, 4]. A stage of heartworm disease was de-
determined for each dog. Results were statistically analyzed and
presented as percentages (qualitative variables) and mean ± standard
deviation (SD) (quantitative variables). In this de-
scriptive retrospective study we defined an incidence of each
radiographic change and scored them, and calculated sizes of a
heart and blood vessels relevant to the HWD among examined
dogs. The most common radiographic changes subjectively
assessed were increased sternal contact (95%) and rounding of
the cranial border (90% of dogs). Scores for subjective assess-
ment of radiographic findings in examined dogs were in the range
2/9 to 5/9 for cardiomegaly and 1/12 to 6/12 for lung pat-
ttern. Average heart sizes measured in LL and DV radiographs
were 10.75 ± 0.78v and 11.04 ± 0.42v, respectively. Measurements
of relevant blood vessels were as follows: vena cava caudalis
0.83 ± 0.10v, right cranial lobar artery 0.31 ± 0.08v and right cau-
dal lobar artery 0.96 ± 0.42v. Further studies are needed to com-
pare these results with results of dogs without HWD in order to
define the most important changes that could be used as a diag-
nostic or prognostic tool. Objectivity in the assessment of the ra-
diographs of dogs with HWD is possible to achieve by scoring
the findings and using objective radiographic measurement.
This work is part of the research done in the project TR31084
granted by the Serbian Ministry of Education and Science.
A27
Occurrence and taxonomical classification of microfilariae in blood samples from canine blood donors localized in south-eastern Poland

Tomczuk Krysztof1, Szczepaniak Klaudiusz2, Grzybek Maciek3, Andrzej Junkuszew2, Paulina Dudko2, Panchev Nicola4, Stefańiak Marzena2, Iwanicki Ryszard4
1Department of Parasitology and Invasive Diseases, University of Life Sciences, Lublin, ul. Akademicka 12, 20-950 Lublin, Poland; 2Faculty of Biology and Animal Breeding Department of Small Ruminants Breeding and Agricultural Advisory, University of Life Sciences In Lublin ul. Akademicka 13 20-950 Lublin, Poland; 3IDEXX Laboratories, 71636 Ludwigsburg, Germany; 4Lubelskie Centrum Małych Zwierząt, ul. Stefczyka 11, 20-151 Lublin, Poland

Correspondence: Tomczuk Krysztof (krysztof.tomczuk@up.lublin.pl)

Parasites & Vectors 2016, 10(Suppl 1):A27

Blood transfusions are routinely performed in small animal veterinary hospitals. However, in many practices a screening of blood donors for canine vector-borne diseases (CVBDs) is not a mandatory procedure. So far dogs have been not tested for the occurrence of microfilariae in most Polish canine blood banks, which indicates lack of available data regarding microfilariosis among canine blood donors. The survey was carried out in the second half of year – between May and December 2015 what corresponds to the highest levels of microfilariae per ml observed in peripheral blood of dogs from Central and Eastern Europe. A total of 350 blood samples from healthy dogs - blood donors, were analyzed using microscopic and biomolecular methods. Microfilaraemic samples were further analyzed by standard PCR methods. Circulating microfilariae were detected in fresh smear in 20 samples with prevalence of 5.7% (3.6-9.0). PCR analysis revealed that, in total 16 out of 20 samples were positive for D. repens while 4 samples were negative. Other filarial species (D. immitis, Acanthocheilonema reconditum, A. dipetalonema dracunculoides) occurring in Europe were not detected in the analyzed material. Canine dirofilariosis has been spreading during the last years in Central Europe countries [1]. D. repens is a dominant causative agent of canine microfilariosis in Poland [2], which was confirmed in our study. Currently a cross-serological survey also revealed a circulating antigen of D. immitis in these geographical areas. In Poland 0.015% dogs were positive for circulating antibodies D. immitis [3]. Despite the fact that dogs cannot infect Dirofilaria spp. via blood transfusion, the risk of spreading the reservoir and possible immune reactions of the host (blood recipient) indicate that screening tests for dirofilariosis are essential.

References
1. Mitterpáková M, Iglódyová A, Čabanová V, Stloukal E, Miklósiová D. Canine dirofilariosis endemic in Central Europe-10 years of epidemiological study in Slovakia. Parasitol Res 2016; 115: 2389–2395.
2. Demiaszkiewicz AW, Polančyczak G, Dürsba K, Pyziel AM, Kuligowska I, Lachowicz J, Sikorski A. The prevalence and distribution of Dirofilaria repens in dogs in the Mazovian Province of central-eastern Poland. Ann Agric Environ Med. 2014; 21: 701–704.
3. Krämer F, Schaper R, Schunack B, Polozowski A, Piekarśka J, Szwedko A, Jodko R, Kowalska D, Schüpbach D, Panchev N. Serological detection of Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato and Ehrlichia canis antibodies and Dirofilaria immitis antigen in a countrywide survey in dogs in Poland. Parasitol Res. 2014; 113: 3229–3239.

A28
Filaroid helminths in mosquitoes from the Danube Delta/Romania and the analysis of these vectors for potential vector competence

Victoria Wimmer1, Angela Monica Ionică2, Carina Zittra2, Natalsha Leitner3, Jan Votyupa3, David Modry4, Andrei Daniel Mihalca5, Hans-Peter Fuehrer1
1Department of Pathobiology, Institute of Parasitology, University of Veterinary Medicine Vienna, 1210 Vienna, Austria; 2Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3-5, Cluj-Napoca, Romania; 3Department of Parasitology, Faculty of Sciences, Charles University, Viničná 7, 12844 Prague, Czech Republic; 4Department of Pathology, Palacky University, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého tr. 1946/1, 612 42 Brno, Czech Republic; 5Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic

Correspondence: Hans-Peter Fuehrer (hans-peter.fuehrer@vetmeduni.ac.at)

Parasites & Vectors 2016, 10(Suppl 1):A28

In the past decades both Dirofilaria immitis and D. repens have spread from historically endemic areas to central and eastern European countries. Several studies have shown that Dirofilaria species are present in the southern and south-eastern areas of Romania [1]. However, information about the vectors in the Danube Delta and their vector competence is lacking. In July 2015 more than 5,000 mosquitoes were collected in the Danube Delta in Romania at various locations (including mosquito traps next to a dog infected with both D. immitis and D. repens). Mosquitoes were classified to species-level using the key after Becker et al. [2]. In one part of the study species-specific mosquitoes were pooled (up to 25 individuals per day/trap/mosquito species). DNA was extracted and the samples were screened for filarial helminths using conventional PCRs. For the second part of the study 300 specified mosquito individuals caught at the trap next to a microfilariaemic dog positive for D. immitis and D. repens were segregated into head/thorax and abdomen prior to DNA extraction. Each thorax/head and abdomen was screened for the presence of filarial DNA separately. All positive PCR products were further analysed by sequencing.

In the Danube Delta, in which the mosquito season is more than 200 days long, mosquito collection started in March up to November. More than 1,000 identified mosquitoes were collected in 30 different traps in 30 different sites distributed along the Danube Delta between Vama Veche and Sulina. Species distribution in the Danube Delta was: Culex pipiens (38%), Culex sitiens (29%), Culex gelidus (17%), Culex quinquefasciatus (10%), Culex argyrostoma (1%), Culex antennatus (1%). Culex pipiens and Culex sitiens are the most significant components of the Danube Delta mosquito fauna.

Culex pipiens and Culex sitiens were screened by nested PCR for the filarial DNA. DNA from the same mosquito species was sequenced and the results were compared with the existing sequences in the GenBank database.

References
1. Ioniţă AM, Matei IA, Mircean V, Dumitrache MO, D’Amico G, Györke A, Panchev N, Annoscia G, Albrechtová K, Otranto D, Modry D, Mihalca AD. (2015) Current surveys on the prevalence and distribution of Dirofilaria spp. and Acanthocheilonema reconditum infections in dogs in Romania. Parasitol Res. 114(3):975-82. doi: 10.1007/s00436-014-4263-4.
2. Becker N, Petrić D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A. Mosquitoes and their control. Berlin: Springer; 2010.

4TH BAYER ANGIOSTRONGYLOSIS FORUM 2016

A29
Angiostrongylus vasorum – what’s new?

Manuela Schryder (manuela.schryder@uzh.ch)

Institute of Parasitology, University of Zurich, 8057 Zurich, Switzerland

Parasites & Vectors 2016, 10(Suppl 1):A29

The increasing number of publications since the turn of the millennium mirrors the growing interest in Angiostrongylus vasorum. The
most recent works have focussed on various aspects of the infection. First of all, the expansion of *A. vasorum* in dogs and in wildlife in Europe seems to persist. New reports include cases in dogs from Belgium, Portugal, Bulgaria and Slovakia, all surrounded by countries where *A. vasorum* had previously been observed. In parallel, studies in wildlife have confirmed that foxes represent the most important reservoir, with prevalences over 70%.

Recent epidemiological studies in foxes and dogs showed that annual precipitation and temperature influenced the distribution of *A. vasorum*, and that in the Alps, altitudes above 700 m asl represent a limiting factor for parasite transmission. Field studies illustrated the variability of spatial distribution and the variability of the slug fauna acting as intermediate hosts, which was suggested to explain the clumpy distribution of *A. vasorum*. An additional confounder may be represented by birds: in addition to previously described frogs, experimental studies have shown that chicken (and therefore potentially other bird species), may also act as paratenic hosts.

In dogs, the classical larval detection in faeces is frequently complemented with PCR performed on different substrates, including bronchoalveolar fluid. Comparisons performed between coproscopic, biomolecular and serological methods testify to the high performance of serological methods. A commercially available test kit for *A. vasorum* antigen detection allows the diagnosis of canine angiostrongylosis within 15 minutes. It also proved highly sensitive when analysing cardiopulmonary tissue fluid of foxes. Last but not least, the broad variety of clinical signs associated with *A. vasorum* infection accounts for an excellent camouflage of the disease, including manifestations in the eyes, neurological disorders, bleeding from various surfaces or internally or even by neutropile dermatitis, hepatic abnormalities or concurrent infections with the heartworm *Dirofilaria immitis*. In clinical patients with respiratory distress the occurrence of pulmonary hypertension was proposed as a negative predictor of survival to the infection Importantly, bleeding seems to occur in up to one third of clinical cases, however results of tests evaluating the coagulation system are not fully consistent and the reasons behind the impaired coagulation are still debated. In conclusion, the clinical diagnosis of angiostrongylosis represents a challenge, therefore disease awareness is pivotal. Moreover, a wide range of open questions remain to be addressed.

**A31**

**Seroprevalence of Angiostrongylus vasorum in Swedish dogs: a national survey**

Gülio Grandi1, Eva Osterman-Lind2, Roland Schaper3, Ulika Forshell4, Manuela Schnyder5

1Department of Microbiology, National Veterinary Institute, Uppsala; 2Bayer Animal Health GmbH, Leverkusen, Germany; 3Bayer HealthCare - Animal Health, Copenhagen, Denmark; 4Institute of Parasitology – University of Zurich, Zurich, Switzerland

**Correspondence:** Gülio Grandi (giulio.grandi@sva.se)

In conclusion, the clinical diagnosis of angiostrongylosis represents a challenge, therefore disease awareness is pivotal. Moreover, a wide range of open questions remain to be addressed.

**A30**

**Angiostrongylus vasorum** in its intermediate hosts: an epidemiological survey in Germany

Malin Lange1, Felipe Penagos1, Carlos Hermosilla1, Roland Schaper2, Anja Taubert1

1Institute of Parasitology, Justus Liebig University, Gießen, Germany; 2Bayer Health GmbH, Leverkusen, Germany; 51368

**Correspondence:** Malin Lange (malin.k.lange@vetmed.uni-giessen.de)

Infections with the French Heartworm *Angiostrongylus vasorum* represent neglected diseases of dogs in Germany. Due to the localization of *A. vasorum* in the right heart and pulmonary artery this parasite causes a multi-factorial disease being represented by general, respiratory, circulatory, bleeding and neurological disorders that occasionally lead to death. Recent European surveys indicate that this parasite is spreading in Europe. Actual data on prevalences in dogs and foxes (acting as reservoir hosts) reveal several endemic foci in Germany. The life cycle of *A. vasorum* is obligatory linked to an intermediate host being represented by a wide range of slugs and snails. Given that actual data on *A. vasorum* infections in intermediate hosts are missing for Germany, we here conducted an epidemiological survey on slugs in selected regions of Hesse and Rhineland-Palatinate. To account for seasonal variations slugs were collected throughout the season in spring, summer, autumn and winter in four different areas (two spots for Hesse and Rhineland-Palatinate, each) that were previously proven to be hyperendemic for *A. vasorum* fox infections. Thus, a total of 2701 slugs were collected and examined for lungworm larvae using the techniques of artificial digestion and microscopy. The confirmation of the lungworm species will be made by specific PCRs. Preliminary data revealed a total *A. vasorum* prevalence of 4.6% in slugs based on microscopic analyses. The number of *A. vasorum* larvae per slug varied considerably (1-546 larvae per specimen). Considering the different sampling areas, some hotspots with relatively high *A. vasorum* prevalences in slugs (up to 10%) were identified. *A. vasorum* prevalences varied with the season since highest prevalences were detected in summer (9.1%), whilst the lowest number of infected slugs was found in winter (0.8%). Besides *A. vasorum*, we additionally detected other lungworm larvae in slug samples: *Crenosoma vulpis/striatum* (lungworm of dog/hedgehog, 2.2%) and *Aeluropostongylus abstrusus* (feline lungworm, 0.2%). Overall, the current data demonstrate that dogs are at a permanent risk for *A. vasorum* infections (even in winter) when living in the investigated areas.

**References**

1. Elsheikha HM, Holmes SA, Wright I, Morgan ER, Lacher DW. Recent advances in the epidemiology, clinical and diagnostic features, and control of canine cardio-pulmonary angiostrongylosis. Vet Res. 2014; 45:52.

2. WA FA. Fransk hjerteorm påvist for første gang i Norge. Website of the Norwegian Veterinary Institute, http://vetinstprot1.prod1.slides.no/eng, accessed 14th of April, 2016.

3. Åslåd, B. Christensson D; Osterman Lind E, Ägren E, Mörten T. Angiostrongylus vasorum etablerade i Sverige. Svensk Veterinär Tidning. 2003; 55:11–15.
A32 Geographical distribution of metascarongylid nematodes Angiostrongylus vasorum and Crenosoma vulpis in Slovak wildlife - preliminary study
Viktória Čabanová, Zuzana Humliková, Martina Miterpáková
Institute of Parasitology, Slovak Academy of Sciences, Košice, 040 01, Slovakia
Correspondence: Martina Miterpáková (miterpak@saske.sk)
Parasites & Vectors 2016, 10(Suppl 1):A32

Angiostrongylus vasorum and Crenosoma vulpis are important lungworms infecting dogs and wild canids, and their incidence is increasing worldwide. In Europe, red fox (Vulpes vulpes) is considered as a major reservoir host of these species. With regard to successful anti-rabies vaccination programmes and their urbanisation, red foxes represent a significant infection risk for dogs. Despite it, data on the occurrence of these parasites in fox populations are very scanty in a lot of European countries. In Slovakia, A. vasorum in dogs was for the first time reported in 2013 and then in 2014 [1, 2]. Consequent serological survey confirmed circulating A. vasorum antigen or the parasite-specific antibodies in 6.22% of dogs investigated [3]. A. vasorum was not previously reported in Slovak red foxes. On the other hand, C. vulpis was noticed in 1960ties and 1980ies in red foxes from Tatra National Park, Northern Slovakia, but its distribution and prevalence rate has never been formally surveyed. Therefore, the aim of the present study was to uncover real occurrence of A. vasorum and C. vulpis in fox populations in Slovakia and estimate the risk of infection for dogs. Between September 2015 and April 2016 faecal samples of 420 red foxes were examined using flotation technique with zinc sulphate and Baermann migration method. The first stage larvae were determined by morphometric and morphological characteristics. Of 420 red foxes, 80 (19.05%) were positive for C. vulpis and 25 (5.95%) for A. vasorum. Only one fox showed dual infection with both species. Geographic information system was used to map the spatial distribution of infected foxes. In conclusion, it should be said, it is the first monitoring of A. vasorum and C. vulpis in Slovak foxes and the data obtained will serve for any future epidemiological researches.

Acknowledgement
The research was supported by the Slovak Grant Agency VEGA, projects No. 2/0018/16.

References
1. Humliková Z, Miterpáková M, Mandelkova V. First autochthonous case of canine Angiostrongylus vasorum in Slovakia. Parasitol Res, 2013; 112: 3505–3508.
2. Miterpáková M, Hurníková Z, Zalewski A.P. The first clinically manifested case of angiostrongylosis in a dog in Slovakia. Acta Parasitol, 2014, 59: 661–665.
3. Miterpáková M, Schnyder M, Schaper R, Hurníková Z, Čabanová V. Serological survey for canine angiostrongylosis in Slovakia. Helminthologia, 2015; 52: 205–210.

A33 Baermann fecal examination survey of dogs showing signs of respiratory disease in Ontario, Canada
Gary Conboy1, Nicole Murphy1, Tamara Hofstede2
1Department of Pathology and Microbiology, Atlantic Veterinary College, Charlottetown, Prince Edward Island, C1A 4P3, Canada; 2Animal Health Bayer Inc, Mississauga, Ontario, L4W 5R6, Canada
Correspondence: Gary Conboy (conboy@upei.ca)
Parasites & Vectors 2016, 10(Suppl 1):A33

Canine respiratory disease due to helminth infection is considered infrequent. Diagnosis is challenging due to poor detection sensitivity of fecal flotation for most species of lungworms. Along with an over-reliance in clinical practice on fecal flotation for detection of parasitism, this leads to the potential for under-diagnosis of lungworms. A further complication is the sporadic fecal larval shedding patterns typical of metascarongylid infections. Fecal samples (3 consecutive day collections) from dogs showing signs of respiratory disease were examined for the presence of lungworm first-stage larvae (L1) or eggs using the Baermann technique and zincsulfate centrifugal flotation from October 2014 to May 2016. Afrebrile dogs showing signs of respiratory disease (mainly chronic cough) that had not received an anthelmintic (except pyrantel or selamectin) within the last 60 days were included in the study. Baermann examinations were done on a 12-gram composite sample (4 grams of feces from each of the 3 collection days) and a 12-gram sample (day 3 collection) for each dog. Larval counts (L1/gram feces = LPG) were done on each of the 3 day collection samples if larvae were detected on either the composite or day 3 sample. Helminths known to cause respiratory disease were detected in 6.9% (22/317) of the samples examined. Duration of clinical signs prior to diagnosis ranged from 14 – 210 days. First-stage larvae of Crenosoma vulpis (4.7%; 15/317), Strongyloides stercoralis (0.6%; 2/317), Filarioidea hirthi/Oslerus osleri (0.3%; 1/317) were detected on Baermann examination. Detection of A. abstrusus L1 in the one dog was considered a spurious finding. Eggs of Paragonimus kellicotti (0.6%; 2/317) and Eucoleus boehmi (0.3%; 1/317) were detected on centrifugal flotation. All of the C. vulpis infections were detected from October to May with nearly half occurring in March. Baermann examination of the 3-day composite sample detected 86.7% (13/15) of C. vulpis infections compared to 73.3% (11/15) by examination of a single (day 3) sample. Larval shedding levels ranged from 0 – 455 LPG (Mn = 22.2 LPG); only 2 dogs shed more than 20 LPG. Lungworm infection should be considered as a possible cause in any case of respiratory disease in dogs in eastern Canada (and likely elsewhere). Three daily Baermann fecal examinations had greater C. vulpis detection sensitivity than a 3-day collection composite and both were superior to examination of a single day collection sample.

A34 Lungworms in Germany 2003 - 2015 - a true increase? Dieter Barutzki1, Viktor Dyachenko2, Roland Schaper1
1Veterinary Laboratory Freiburg, Freiburg, Germany, 79108; 2Bayer Animal Health GmbH, Leverkusen, Germany, 51368
Correspondence: Dieter Barutzki (barutzki@labor-freiburg.de)
Parasites & Vectors 2016, 10(Suppl 1):A34

In recent years, infections with Angiostrongylus vasorum in dogs have increasingly been reported in European countries. For some time occurrence and distribution of A. vasorum seemed to be largely confined in isolated endemic foci. New reports of cases in dogs in endemic areas and data of post mortem surveys of foxes in areas previously believed to be free from infections suggest that A. vasorum has increased in prevalence and is spreading geographically within Europe. In Germany only few epidemiological studies have been performed and data on changes in the lungworm distribution in dogs in Germany are lacking. The aim of this study was to present actual data on occurrence and regional geographical distribution of A. vasorum and C. vulpis in dogs in Germany and to analyse these data in terms of evidence for geographically spreading of lungworms in Germany. In a retrospective study, the results of parasitological examinations of faecal samples, which had been submitted to the Veterinary Laboratory Freiburg, from 54,934 dogs between 2003 and 2015 in Germany were analysed. All faecal samples were obtained from privately owned dogs presented to local veterinary surgeons from all parts of Germany for mostly unknown clinical problems, routine examination and animal vaccination or general health check. All specimens were tested by a standardised flotation method with a saturated salt solution and examined by Baermann funnel technique to detect first-stage larvae (L1) of lungworms. The collected data were analysed by a geographic information system (GIS) using the programme RegioGraph 10 (GIK GeoMarketing, Bruchsal) to visualise the regional distribution of A. vasorum and C. vulpis. Rates of infection with A. vasorum and C. vulpis and their geographical distribution were analysed and proved
A35

A coprological and serological survey on Angiostrongylus vasorum in Southern Belgium

Laetitia Lempereur1, Ludovic Martinelle2, Calliste Bayrou3, Françoise Marechal1, Anne-Catherine Dalemans1, Bertrand J Losson1,4

1University of Liège, Faculty of Veterinary Medicine, Laboratory of Parasitology and Parasitic Diseases, Liège, Belgium; 2University of Liège, Faculty of Veterinary Medicine, Experimental Station CARE – FePex, Center for Fundamental and Applied Research for Animal and Health (FARAH), Liège, Belgium; 3University of Liège, Faculty of Veterinary Medicine, Laboratory of Pathology, Liège, Belgium; 4Bayer Health Care, Diegem, Belgium

Correspondence: Bertrand J Losson (blosson@ulg.ac.be)

Parasites & Vectors 2016, 10(Suppl 1):A35

Despite the fact that epidemiological models indicate that Belgium has a favourable climate for the completion of A. vasorum life cycle [1], the parasite was not recorded in this country until 2013 [2]. The aim of the present study was to gain additional information on the distribution and prevalence of A. vasorum infection in dogs through the combined used of in-house detection of circulating specific antigen and coprology. The survey was conducted from November 2014 until February 2016. Seventeen practices were selected across Southern Belgium. Samples were collected from dogs belonging to two populations: a first random dog population (called « control, thereafter) presented for unrelated conditions whereas the second population included dogs showing clinical signs compatible with angiostrongylosis. These two populations were selected based on the absence of travel history outside Belgium during the 3 previous months. Blood samples were collected and an in-clinic serological test detecting A. vasorum circulating Ag (Angio Detect™, IDEXX) was used for initial screening. Stools were collected on 3 consecutive days from dogs with a positive serological screening and examined with the Baermann technique [3]. This was not always possible and in some cases stools were obtained only once or twice. A total of 979 dogs were enrolled. Seven hundred fifty-seven were included in the control group whereas 222 dogs had clinical signs compatible with angiostrongylosis. The distribution of samples according to the different tests is given in Table 1.

Table 1 Distribution of samples according to the different tests (serology versus coprology)

| Test Type     | Control dogs (n=757) (%) | Symptomatic dogs (n=222) (%) | Total (n=979) (%) |
|---------------|--------------------------|-----------------------------|-----------------|
| Angio detect™ IDEXX + | 27 (3.4)                 | 19 (8.6)                    | 46 (4.7)        |
| Angio detect™ IDEXX + and Baermann + | L1 A. vasorum | 7 (1.0) | 6 (2.7) | 13 (1.3) |
|               | L1 C. vulpis             | 1 (0.45)                    | 1 (0.1)         |
|               | L1 A. vasorum and C. vulpis | 2 (0.9) | 2 (0.9) | 2 (0.2) |
| Angio detect™ IDEXX + and Baermann − | 17 (8)                  | 8 (3.6)                     | 25 (2.5)        |
| Angio detect™ IDEXX + and Baermann not performed | 3 (1.5) | 2 (0.9) | 5 (0.5) |
| Angio detect™ IDEXX − and Baermann + | L1 A. vasorum | 1 (0.4) | 1 (0.45) | 1 (0.1) |
|               | L1 C. vulpis             | 1 (0.4)                     | 1 (0.1)         |

References
1. Jolly S, Poncelet L, Lempereur L, Caron Y, Bayrou C, Cassart D, Grimm F, Losson B. First report of a fatal autochthonous canine Angiostrongylus vasorum infection in Belgium. Parasitol. Int. 2014, 64: 97–99.
2. Morgan E, Jeffreies R, Krajewski M, Ward P, Shaw S. Canine pulmonary angiostrongylosis. The influence of climate on parasite distribution. Parasitol. Internat., 2009, 58: 406–410.
3. Elsheikha H, Holmes S, Wright I, Morgan E, Lacher D. Recent advances in the epidemiology, clinical and diagnostic features, and control of canine cardio-pulmonary angiostrongylosis. Vet. Res., 2014, 45: 92–103.
Angiostrongylus vasorum is a lungworm infecting dogs, foxes and few other wild carnivores [1-3]. Reports of A. vasorum in dogs increased in the last two decades and foxes were frequently indicated as the relevant parasite reservoir, together with snails acting as intermediate hosts [4-8]. Our aim was to investigate the prevalence, worm burden and regional distributions in Swiss red foxes, as well as to evaluate enzyme-linked immunosorbent assays (ELISA) for detection of circulating A. vasorum antigen and specific antibodies, which had previously been developed for dogs [9, 10]. Over the past five years lungs and hearts of 377 Swiss foxes were examined for the presence of A. vasorum and other lungworms. Blood collected from these foxes was used to evaluate the ELISAs. In the investigated fox population, A. vasorum, Capillaria aerophila and Crenosoma vulpis were identified. C. aerophila was found in all investigated cantons, whereas A. vasorum and C. vulpis did not occur in the canton of Graubünden. Overall prevalence of A. vasorum over the last five years was 45.1% (worm burden: WB 1-44, mean 7.1), increasing from 20.5% in 2012 to 72.3% in 2016, while overall prevalence of A. aerophila and C. vulpis was 63.7% (WB: 1-99, mean 3.2) and 9.0% (WB: 1-48, mean 1.2), respectively. The ELISAs for detection of circulating antigen and specific antibodies had a sensitivity and specificity of 91.2% and 80.5%, respectively. The ELISA for detection of circulating antigen of A. vasorum in serum samples of naturally and experimentally infected dogs. Veterinary Parasitology. 2011; 179(1-3): 152–158.

References
1. Segovia JM, Torres J, Miquel J, Llanesa L, Felis C. Helminths in the wolf, Canis lupus, from north-western Spain. Journal of Helminthology. 2001; 75(2): 183–192.
2. Bourque A, Whitney H, Conboy G. Angiostrongylus vasorum infection in a coyote (Canis latrans) from Newfoundland and Labrador, Canada. Journal of Wildlife Diseases. 2005; 41(4): 816–819.
3. Takács L, Szabó L, Juhász L, Lanszki J, Takács P, Heltai M. Data on the A. vasorum prevalence from year to year. The Veterinary J. 2009;179: 348–359.
4. Morgan ER, Shaw SE, Brenniman SF, De Waal TD, Jones BR, Mulcahy G. Angiostrongylus vasorum: a real heartbreaker. Trends Parasitol. 2005; 21: 49–51.
5. Lurati L, Deplazes P, Hegglin D, Schnyder M. Seroepidemiological survey from 20.5% in 2012 to 72.3% in 2016, while overall prevalence of A. vasorum in dogs increased in the last two decades and foxes were frequently indicated as the relevant parasite reservoir, together with snails acting as intermediate hosts [4-8]. Our aim was to investigate the prevalence, worm burden and regional distributions in Swiss red foxes, as well as to evaluate enzyme-linked immunosorbent assays (ELISA) for detection of circulating A. vasorum antigen and specific antibodies, which had previously been developed for dogs [9, 10]. Over the past five years lungs and hearts of 377 Swiss foxes were examined for the presence of A. vasorum and other lungworms. Blood collected from these foxes was used to evaluate the ELISAs. In the investigated fox population, A. vasorum, Capillaria aerophila and Crenosoma vulpis were identified. C. aerophila was found in all investigated cantons, whereas A. vasorum and C. vulpis did not occur in the canton of Graubünden. Overall prevalence of A. vasorum over the last five years was 45.1% (worm burden: WB 1-44, mean 7.1), increasing from 20.5% in 2012 to 72.3% in 2016, while overall prevalence of A. aerophila and C. vulpis was 63.7% (WB: 1-99, mean 3.2) and 9.0% (WB: 1-48, mean 1.2), respectively. The ELISAs for detection of circulating antigen and specific antibodies had a sensitivity and specificity of 91.2% and 80.5%, respectively. The ELISA for detection of circulating antigen of A. vasorum in serum samples of naturally and experimentally infected dogs. Veterinary Parasitology. 2011; 179(1-3): 152–158.

References
1. CHAPMAN PS, BOAG AK, GUITIÁN J, BOSWOOD, A. Angiostrongylus vasorum infection in 23 dogs (1999 – 2002). J Small Anim Pract 2004, 45: 435–440.
2. KOCH J, WILLESEN JL. Canine pulmonary angiostrongylosis: An update. Veterinary Parasitology. 2013; 207(3): 355–358.
3. MORGAN ER, SHAW SE, BRENNIMAN SF, DE WAAL TD, JONES BR, MULCAHY G. Angiostrongylus vasorum: a real heartbreaker. Trends Parasitol. 2005; 21: 49–51.
4. TAUBERT A, PANTCHEV N, VRHOVEC MG, BAUER C, HERMOSILLA C. Lungworm infections (Angiostrongylus vasorum, Crenosoma vulpis, Aelurostrongylus abstrusus) in dogs and cats in Germany and Denmark in 2003-2007. Vet Parasitol 2009, 159: 175–180.
5. LURATI L, DEPLAZES P, HEGGLIN D, SCHNYDER M. Seroepidemiological survey from 20.5% in 2012 to 72.3% in 2016, while overall prevalence of A. vasorum in dogs increased in the last two decades and foxes were frequently indicated as the relevant parasite reservoir, together with snails acting as intermediate hosts [4-8]. Our aim was to investigate the prevalence, worm burden and regional distributions in Swiss red foxes, as well as to evaluate enzyme-linked immunosorbent assays (ELISA) for detection of circulating A. vasorum antigen and specific antibodies, which had previously been developed for dogs [9, 10]. Over the past five years lungs and hearts of 377 Swiss foxes were examined for the presence of A. vasorum and other lungworms. Blood collected from these foxes was used to evaluate the ELISAs. In the investigated fox population, A. vasorum, Capillaria aerophila and Crenosoma vulpis were identified. C. aerophila was found in all investigated cantons, whereas A. vasorum and C. vulpis did not occur in the canton of Graubünden. Overall prevalence of A. vasorum over the last five years was 45.1% (worm burden: WB 1-44, mean 7.1), increasing from 20.5% in 2012 to 72.3% in 2016, while overall prevalence of C. aerophila and C. vulpis was 63.7% (WB: 1-99, mean 3.2) and 9.0% (WB: 1-48, mean 1.2), respectively. The ELISAs for detection of circulating antigen and specific antibodies had a sensitivity and specificity of 91.2% and 89.4%, and of 42.2% and 92.0%, respectively. Cross-reactions with other parasite species were very limited. We therefore present reliable and quick serological methods to detect A. vasorum in foxes and conclude that A. vasorum is established in the Swiss fox population with increasing prevalence from year to year.
The adult stages of the nematode *Angiostrongylus chabaudi* (Strongylida, Angiostrongylidae), parasitize the pulmonary arteries and right ventricle of the heart and have been reported in wildcats (*Felis silvestris silvestris*) in Italy, in 1957 [1]. Since that first description, *A. chabaudi* has never been reported, with the exception of the recent descriptions of immature stages in two cats in Italy [2, 3]. The case presented here is an infection by *A. chabaudi* in a wildcat from Northern Greece. The wildcat was found road-killed near the lake Kerkini (Macedonia, Greece). During necropsy, nematode parasites were found in the right ventricle of the heart and the pulmonary artery. The parasites were adult males and females and according their morphological characteristics were identified as *A. chabaudi* (Fig. 6). Additionally, parasitological examination of faeces and bronchoalveolar lavage revealed the presence of first stage larvae (L1) measuring 362-400 × 15-18.5 μm, with a kinked tail presenting a dorsal spine and a notch (Fig. 7). Both adults and larvae were subjected to molecular examination that confirmed that the parasites belong to the species *A. chabaudi*. The finding of histopathological examination of the lungs included heavy, extended, interstitial granulomatous pneumonia, with lesions detected around the larvae and eggs of the parasite. These findings were most likely, exclusively due to the presence of *A. chabaudi*, as there were no other parasites found in the lungs, suggesting that this parasite can be quite pathogenic to its hosts. The first description ever [4] of *A. chabaudi* L1 provides the necessary evidence that this nematode can complete its life cycle in the European wildcat, which should be considered its definitive host. The complete life cycle of the parasite remains unknown. For this reason, investigations that will include identification of intermediate hosts (most likely terrestrial molluscs), and development of the parasite both in the vertebrate and invertebrate host, are needed. The description of the diagnostic stage (L1) of *A. chabaudi* provides the basic information for future studies that will investigate infection in other feline species, e.g. the domestic cat and the implications to their health status. It is important to monitor in what extent can *A. chabaudi* affect domestic cats, a scenario that is possible but seems sporadic, according the recent available information of immature, unfertilized, not fully developed parasites isolated from domestic cats.

**References**

1. Biocca E. *Angiostrongylus chabaudi* n. sp. parasita del cuore e dei vasi polmonari del gatto selvatico (*Felis silvestris*). R. Accad. Naz. Lincei. 1957, 22: 526–532.
2. Varcasia A, Tamponi C, Brianti E, Cabras PA, Boi R, Pipia AP, Giannelli A, Otranto D, Scala A. *Angiostrongylus chabaudi* Biocca, 1957: a new parasite for domestic cats? Parasit Vectors., 2014, 7: 588.
3. Traversa D, Lepri E, Veronesi F, Pacletti B, Simonato G, Diaferia M, Di Cesare A. Metastrongyloid infection by *Aelurostrongylus abstrusus*, *Troglostrongylus brevior* and *Angiostrongylus chabaudi* in a domestic cat. Int. J. Parasitol., 2015, 45: 685–690.
4. Diakou A, Psalla D, Migli D, Di Cesare A, Youlatos D, Marcé F, Traversa D. First evidence of the European wildcat (*Felis silvestris silvestris*) as definitive host of *Angiostrongylus chabaudi*. Parasitol. Res., 2016, 115: 1235.
Angiostrongylus chabaudi and A. daskalovi in wild carnivores from Romania

Călin M Gherman 1, Georgiana Deal 1, Angela M Ionica 1, Gianluca D’Amico 1, Domenico Otranto 1, Andrei D Mihalca 1, Domenico Otranto 1 1Department of Parasitology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăștur 3-5, 400372, Cluj-Napoca, Romania; 2Department of Medicine Veterinaria, Universität degli Studi di Bari, Bari, Italy

Correspondence: Andrei D Mihalca (amihalca@usamvcluj.ro)

Parasites & Vectors 2016, 10(Suppl 1):A40

Angiostrongylus chabaudi is a rare feline cardiac-pulmonary nematode, described in 1957 in a wildcat from Italy and reported subsequently in domestic cats from Italy and wildcats from Greece. Similarly, A. daskalovi is a cardio-pulmonary nematode of mustelids described in Bulgaria in 1988 and later reported also in badgers from Spain. The present study reports A. chabaudi and A. daskalovi, in wildcats (Felis silvestris) and badgers (Meles meles), respectively, collected as roadkills in Romania. After careful morphological and morphometrical identification, the partial mitochondrial cytochrome c oxidase subunit 1 (cox 1) gene and the internal transcribed spacer 2 (ITS2) of the rRNA gene were sequenced and compared with sequences deposited in GenBank. This study reports for the first time in Eastern Europe the presence of A. chabaudi and for the first time in Romania the presence of A. daskalovi, bringing new insights in their SEM ultrastructure and molecular identification.

POSTER SESSION

A41

First characterization of haemocyte extracellular traps in gastropods induced by Angiostrongylus vasorum, Crenosoma vulpis, Aelurostrongylus abstrusus and Troglotrystrongylus brevieri

Malin Lange 1, Felipe Penagos 1, Tamara Muñoz-Caro 1, Gerd Magdziak 1, Uwe Gättner 2, Helena Mejer 3, Roland Schaper 4, Carlos Hermosilla 1, Anja Taubert 1

1Institute of Parasitology, Justus Liebig University, Giessen, Germany; 2Institute of Anatomy and Cell biology, Justus Liebig Universität, Giessen, Germany; 3Parasitology and Aquatic Diseases, University of Copenhagen, Copenhagen, Denmark; 4Bayer Health GmbH, Leverkusen, Germany. 513689

Correspondence: Malin Lange (malin.k.lange@vetmed.uni-giessen.de)

Parasites & Vectors 2016, 10(Suppl 1):A41

In the last years lungworm infections of canids and felids have been the focus of special attention due to their emergence in several countries and spread into non-reported areas [1, 2, 3]. Slugs and snails have been reported as intermediate hosts of these metastastrongyloid species [4, 5]. Haemocytes, a denomination of cell types in invertebrates that freely circulate in the haemolymph, are involved in several physiological functions like coagulation and innate immune response [5]. Haemocytes are similar to mammalian phagocytes, able to produce Extracellular Traps [6]. This phenomenon, denominated ETosis, consists in a programmed cell death form in which the chromatin and antimicrobial proteins are expelled into the extracellular region and finally induce the formation of fiber-like structures, that have the capacity to trap and inactivate pathogens of diverse kinds, like bacteria, viruses and others parasites [9]. Recently the ETosis mechanism was characterized in invertebrates [8, 10]. The aim of this study was to characterize for the first time gastropod's Haemocytes Extracellular Traps (HETs) induction and formation against metastastrongyloid larvae. Haemocytes from the slugs species Arion lusitanicus and Limax maximus, and the Giant African Snail Achatina fulica, were cultured with Angiostrongylus vasorum, Crenosoma vulpis, Aelurostrongylus abstrusus and Troglotrystrongylus brevieri L1 larva as well as L3 larvae of A. vasorum at room temperature (±20 °C) and the incubation time varied between 30 min. to 24 h. The visualisation of the HETs was performed using phase contrast microscopy, scanning electron microscopy and fluorescence microscopy. Confronting gastropod haemocytes with the above mentioned species of lungworm larvae revealed in the phase contrast microscopy that L1 and L3 got entangled with a non-defined material originating from the haemocytes. These delicate non-defined ET-like structures were examined in more detail with the technique of SEM imaging. This method renewed our strong suspicion that these structures represent gastropod-derived ETs. Immunofluorescence microscopy revealed that these structures contain histones and DNA. Which have been proven to play an important role in the process of ETosis [11, 12]. This survey represents first indications on slugs and snails casting HETs. All methods used to visualize Extracellular Trap-formation provided strong evidence that this innate immune defence mechanism also exists in gastropods.

References

1. Helm, J., Roberts, L., Jeffries, R., Shaw, S. E., Morgan, E. R. Epidemiological survey of Angiostrongylus vasorum in dogs and slugs around a new endemic focus in Scotland. The Veterinary record. 2015; 177
2. Taubert, A., Pantchev, N., Vrhavec, M.G., Bauer, C., Hermosilla, C. Lungworm infections (Angiostrongylus vasorum, Crenosoma vulpis, Aelurostrongylus abstrusus) in dogs and cats in Germany and Denmark in 2003-2007. Veterinary parasitology. 2009; 159:175–180.
3. Traversa, D., Di Cesare, A., Conboy, G. Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. Parasites & vectors. 2010; 62: 62.
4. Ferdushy, T., Hasan, M.T. Angiostrongylus vasorum: the 'French Heartworm'. Parasitology research. 2010; 107:765-771.
5. Patel, Z., Gill, A. C., Fox, M. T., Hermosilla, C., Backeljau, T., Breugelmans, K., Elson-Riggins, J. G. Molecular identification of novel intermediate host species of Angiostrongylus vasorum in Greater London. Parasitology research. 2014; 113:4363–4369.
6. Yoshino, T. P., Wu, X. J., Gonzalez, L. A., & Hokke, C. H. Circulating Biophanalaria glabrata haemocyte subpopulations possess shared schistosome glycans and receptors capable of binding larval glycoconjugates. Experimental parasitology. 2013; 133: 28–36.
7. Cheng, T. C., & Sullivan, J. T. Effects of heavy metals on phagocytosis by molluscan haemocytes. Marine Environmental Research. 1984; 14:305–315.
8. Robb, C. T., Dyrindra, E. A., Gray, R. D., Rossi, A. G., & Smith, V. J. Invertebrate extracellular phagocyte traps show that chromatin is an ancient defence weapon. Nature communications. 2014; 5.
9. Brinkmann, V., Goossen, C., Kühr, L. I. & Zychlinsky. A. Automatic quantification of in vitro NET formation. Frontiers in Immunology. 2012; 3.
10. Poirier, A. C. Schmitt, P., Rosa, R. D., Vanhove, A. S., Kliefer-Jaquindon, S., Rubio, T. P., Destoumieux-Garzón, D. Antimicrobial Histones and DNA Traps in Invertebrate Immunity: Evidences in Crossostegia Gigas. Journal of Biological Chemistry. 2014; 289:24821–24831.
11. Brinkmann, V., U. Reichard, C. Goossen, B. Fauler, Y. Uhlemann, D.S. Weiss, Y. Weinrauch, A. Zychlinsky. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532–1535.
12. Urban, C.F., D. Ermert, M. Schmid, U. Abu-Abed, C. Goosmann, W. Nacken, V. Brinkmann, P.R. Jungblut, A. Zychlinsky. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog. 2009; 5

A42

Assessment of recovery rates and morphology of larvae A. abstrusus in flotation methods using five solutions with different specific gravities (S.G.)

Klaudiusz Szczepaniak 1, Krzysztof Tomczuk 1, Maciej Grzybek 1, Ryszard Iwanicki 2

1Department of Parasitology and Invasive Diseases, University of Life Sciences, Lublin, ul. Akademicka 12, 20-950 Lublin, Poland; 2Lubelskie Centrum Małych Zwierzy, ul. Stefczyka 11, 20-151 Lublin, Poland

Correspondence: Klaudiusz Szczepaniak (k.o.szczepaniak@up.lublin.pl)

Parasites & Vectors 2016, 10(Suppl 1):A42

The most prevalent cardiopulmonary nematodes in domestic cats in Europe is Aelurostrongylus abstrusus [1]. Recently, Baermann migration method is the gold standard for the diagnosis of lungworm invasions, but takes 24 h and requires at least 4 g of fresh feral samples. Furthermore, taxonomical classification of motile larvae may
be difficult, because their identification is particular based on the tail shape [2]. Unlike the Baermann method flotation-based techniques are easy to performed, fast and allow to detected wide range of parasites. Fecal samples could be delivered to the laboratory preserved or frozen. Specific gravities of different flotation fluid as well as exposures time resulted in the number and morphologic deformations of the larvae derived from each methods modification [3]. The aim of this study was to assessment of the recovery rates and morphology of larvae A. abstratus in flotation methods using five fluids with different specific gravities (SG). Fresh fecal sample (6 g) from natural infected with A. abstratus cat (three years old, male, previously not treated) were obtained. The number of lungworm larvae per gram of feces (LPG) was estimated - 2800/g, using modified Baermann methods and McMaster chambers. Subsequently, five flotation with different flotation solutions respectively: 33% ZnSO4 (SG 1.18), saturated NaCl (SG 1.20), commercially available NaNO3 - Fecasol (SG 1.20), saturated NaCl and saccharose (SG 1.25), saturated NaNO3 (SG 1.33) were performed. We used the following procedure: 1 g fecal sample was mixed with 35 ml flotation solution and poured through a strainer into a (25 ml) glass Erlenmeyer Flask. Slides were directly analyzed under light microscope with Nomarski contrast. A total number of larvae (recovery rates) for each flotation was estimated. Simultaneously larvae were recorded as identifiable (tail was visible) or unidentifiable (tail was not visible e.g., morphologic deformations or curled larvae). Statistical data analysis was performed using Analysis ToolPak Microsoft Office Excel. The larvae of A. abstratus were found in all flotations. The biggest recovery rates 3.2 and 3.1 were achieved using flotation solutions with the highest specific gravities (saturated solutions of: NaCl/Saccharose and NaNO3). In solutions with SG from 1.18 to 1.2 number of detected larvae were lower but their characterized by high percentage of identifiable larvae ranged from 56.3% (saturated NaCl), 71.4% (Fecasol) to 85.7% (33% ZnSO4). Details of results are presented in Table 2.

References
1. Barutzki D, Schaper R. Occurrence and regional distribution of Aelurostrongylus abstrusus in cats in Germany. Parasitol Res 2013; 112: 255-261.
2. Ribeiro VM, Lima WS. Larval production of cats infected and re-infected with Aelurostrongylus abstrusus (Nematoda: Protostrongylidae). Rev Med Vet. 2001; 152: 815-820.
3. Lima VF, Cringoli G, Rinaldi L, Monteiro MF, Calado AM, Ramos RA, Meira-Santos PO, Alves LC. A comparison of mini-FLOTAC and FLOTAC with classic methods to diagnosing intestinal parasites of dogs from Brazil. Parasitol Res 2015; 114: 3529-3533.

Table 2 Comparison of various flotation fluids in A. abstrusus larvae (L1) detection
| Flotation solutions - specific gravities | Recovery rates for LPG, 2800 | Number of larvae in flotation | Identifiable % | Unidentifiable % | Average length of larvae |
|----------------------------------------|-------------------------------|------------------------------|----------------|------------------|-------------------------|
| 33% ZnSO4 (SG 1.18)                    | 0.5                           | 14                           | 12             | 2                | 85.7% 340.6              |
| saturated NaCl (SG 1.20)               | 1.1                           | 32                           | 18             | 14               | 56.3% 354.9              |
| commercially available NaNO3 - Fecasol (SG 1.20) | 1.0                       | 28                           | 20             | 8                | 71.4% 359.2              |
| saturated NaCl and saccharose (SG 1.25) | 3.2                       | 90                           | 28             | 62               | 31.1% 374.2              |
| saturated NaNO3 (SG 1.33)              | 3.1                           | 86                           | 20             | 66               | 23.3% 383.8              |

A43
A ten-year retrospective study of angiostrongylosis at Alfort Veterinary School, Ile de France
Benjamin Bedel 1, Radu Blaga 2, Vassilili Gouni 3, Valérie Cherbuli 1, Ghita Benchekroun 3, Stéphane Blot 1, Patrick Verwaerde 1, Bruno Polack 4
1 Department of Emergency and intensive care, Centre Hospitalier Universitaire, Université Paris-Est, Ecole Nationale Vétérinaire d’Alfort, 94704 Maisons-Alfort, France; 2 Department of parasitology, BioPôle, Université Paris-Est, Ecole Nationale Vétérinaire d’Alfort, 94704 Maisons-Alfort, France; 3 Department of Cardiology, Centre Hospitalier Universitaire, Université Paris-Est, Ecole Nationale Vétérinaire d’Alfort, 94704 Maisons-Alfort, France; 4 Department of Small Animal Internal Medicine, Centre Hospitalier Universitaire, Université Paris-Est, Ecole Nationale Vétérinaire d’Alfort, 94704 Maisons-Alfort, France
Correspondence: Bruno Polack (bruno.polack@vet-alfort.fr)
Parasites & Vectors 2016, 10(Suppl 1):A43

A retrospective study was conducted for the period of 2005-2014, on the identified cases of angiostrongylosis within the clinics of Alfort Veterinary School which receive around 9000 dogs per year. During the ten-year period, the research for angiostrongylosis was done on 804 animals aging from 2 month to 18 year old (mean age = 5.6 years). Three different parasitological methods were performed: faecal examination by Baermann technic (mainly on faeces of three consecutive days), broncho-alveolar lavage (BAL) direct observation by binocular microscopy and Angiostrongylus antigen detection test (IDEXX Angio Detect™ Test; used only in 2014), respectively on 718, 150 and 3 dogs. Some dogs were tested by two different methods. Infection by Angiostrongylus vasorum was detected in thirty dogs, corresponding to 3.7% of tested animals. Infected dogs were aged from 4 months to 16-year-old (mean age = 5.9 years). Positive results were observed on 30 Baermann tests (4.2% of positive) and 1 BAL examination (0.7%, this animal was also tested by Baermann). Concerning the annual dynamic of identified cases, except for 2009, 2010 and 2014, when respectively 5, 4 and 1 case have been identified, for the rest of the period, an annual 3 cases identification rate was observed. The detection was higher during the first 5 months of the year, 5.4% versus 2.3% for the last 7 months. The clinical observed symptoms were very variable: dyspnoea, coagulopathy, right-sided heart failure, cutaneous larva migrans.

A44
Prevalence of Aelurostrongylus abstrusus in Danish cats
Alice P. Hansen 1, Lene M. Vinther 1, Line K. Skarbye 1, Caroline S. Olsen 2, Helena Mejer 1, Jakob L. Willesen 1
1 Department of Veterinary Disease Biology, University of Copenhagen, 1870 Frb C., Denmark; 2 Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, 1870 Frb C., Denmark
Correspondence: Jakob L. Willesen (jw@sund.ku.dk)
Parasites & Vectors 2016, 10(Suppl 1):A44

Aelurostrongylus abstrusus is considered the most prevalent lungworm worldwide in domesticated cats [1, 2]. High prevalence rates have especially been found in southern Europe [3, 4] and studies have indicated that the infection is of clinical relevance [5, 6, 7]. A recent study revealed a high occurrence of A. abstrusus in euthanized cats from eastern Denmark [8] which raised concern of an underestimated national prevalence. Based on these findings, the objective of the present study was to investigate the national prevalence of A. abstrusus in Danish cats. For this purpose, faecal samples from 327 cats were collected between August and October 2015. The study population consisted primarily of outdoor cats from shelters distributed across Denmark and a modified Baermann method was used to test for the infection. The national prevalence of A. abstrusus was 8.3% [95% CI: 5.6-11.9] with substantial regional variation. In Northern Jutland the prevalence was 0% [95% CI: 0.0-8.8%] while a prevalence of 31.4% [95% CI: 16.9-49.3] was found in Western Jutland. The prevalence in the remaining regions varied from 4.5-9.7%.
In dogs *Angiostrongylus vasorum* often causes a severe infection characterized by varying signs similar to those of other canine diseases. Although this parasitosis may be life-threatening, dog angiostrongylosis is often underdiagnosed and veterinarians do not use appropriate diagnostic tests. Six cases of angiostrongylosis are here described, with a focus on their clinical features, that were unusual and confounding. Although the six were referred with clinical signs that may occur in the infection by *A. vasorum*, the animals were suspected to have other diseases before a correct diagnosis was achieved. Case 1: a dog showed clinical, radiographic and ultrasonic features compatible with a pulmonary tumour; case 2: this dog was simultaneously infected by *A. vasorum* and *Dirofilaria immitis* but the former nematode was not included in the differential diagnosis; case 3: a critically ill dog was referred for a severe and then fatal dyspnoea of initially unknown origin; case 4: a thrombocytopenia recorded in a dog with hemorrhages and ecchymoses was erroneously attributed to an inherited, immune-mediated or infective origin; Case 5: a discospondylitis was considered to be the cause of neurological signs in a dog; case 6: a cardio-pulmonary dirofilariosis was diagnosed in a dog that had, on the contrary, only angiostrongylosis. A prompt administration of a parasiticide (in most cases topical moxidectin) was efficacious in the treatment of *A. vasorum* infection in dogs n. 1, 2, 4 and 5, i.e. those animals that did not show hazardous lung haemorrhages yet at the referral. Currently, canine angiostrongylosis is spreading in various regions for different biological and epidemiological factors. Importantly, animals n. 1-5 were diagnosed with *A. vasorum* in Italian regions where this parasite is not considered endemic. Thus, it is of importance that practitioners must include *A. vasorum* in the differential diagnosis of any clinical picture compatible with dog angiostrongylosis also when the parasite is not expected to occur and/or in the presence of compatible signs even if the clinical picture is atypical. These clinical cases are described in detail in ref. [1].

**References**

[1] Di Cesare A, Traversa D, Manzocchi S, Meloni S, Grillotti E, Auriemma E, Paparrinini F, Garofani C, Ibbi F, Venco L. Elusive *Angiostrongylus vasorum* infections. Parasit Vectors. 2015; 8:438.

**A46** First report of *Angiostrongylus vasorum* infections in dogs as well as in the neozoon intermediate host (*Achatina fulica*) in Medellin, Colombia

Felipe Penagos 1,2, Jesus Gutiérrez 1, Juan D. Velz 1, Diego Piedrahita 1, Malin Lange 1, Carlos Hermosilla 2, Anja Taubert 1, Jenny Chaparro 1

1CIBAV research group, Veterinary Medicine School, Faculty of Agrarian Sciences, University of Antioquia, Medellin, Antioquia, 050034, Colombia;
2Institute of Parasitology, Justus Liebig University Giessen, Giessen, 35392, Germany

**Correspondence:** Felipe Penagos (Felipe.penagos@udea.edu.co)

Parasites & Vectors 2016, 10(Suppl 1):A46

*Angiostrongylus vasorum* is considered as one of the most pathogenic species of the cardiopulmonary system of wild and domestic canids worldwide. As such, in 1961 this metastrongyloid parasite was detected in South America in crab-eating foxes (*Cerdocyon thous*) in Colombia and in domestic dogs in Brazil. These reports in demonstrated clearly the presence of this parasite in South America. Nonetheless, since then very little has been published on *A. vasorum* infections neither in wild canids nor domestic dogs in South America and Colombia. Thus, aim of this study was to gain current knowledge on the presence of *A. vasorum* in domestic dogs as well as neozoon intermediate hosts by analysing dog faecal samples collected in public parks in Medellin city and from collected neozoon terrestrial snails (*Achatina fulica*). In total 364 faecal samples were collected from February to April 2016, analysed thereafter by Bearmann funnel test with submission of the samples for at least 24 h. Additionally 300 *A. fulica* snails were collected and digested for the presence of *A. vasorum* larvae. The larvae obtained from molluscs were identified on the basis of

**References**

[1] Di Cesare A, Traversa D, Manzocchi S, Meloni S, Grillotti E, Auriemma E, Paparrinini F, Garofani C, Ibbi F, Venco L. Elusive *Angiostrongylus vasorum* infections. Parasit Vectors. 2015; 8:438.

**A45** Unusual clinical cases of *Angiostrongylus vasorum* infections

Angela Di Cesare 1, Luigi Venco 2, Simone Manzocchi 3, Eleonora Grillotti 1,4, Edoardo Auriemma 3, Fabrizio Paparrinini 3, Cecilia Garofani 3, Fabrizio Ibbi 3, Donato Traversa 3

1Faculty of Veterinary Medicine, University of Teramo, 64100, Teramo, Italy; 2Veterinary Hospital “Città di Pavia”, Viale Cremona 179, 27100, Pavia, Italy; 3Novara Day Lab – IDEXX Laboratories Italia Granozzo con Monticello, Italy; 4Veterinary Practice “Centro Italia”, Viale Biancifiori 3, 02100, Rieti, Italy; 5Istituto Veterinario di Novara, S.P. 9, 28000, Granozzo con Monticello, Italy; 6Bayer Sanità Animale, Viale Certosa 130, 20156, Milan, Italy; 7Veterinary Practice “Poggio dei Pini” Strada 40, 09012, Capoterra, Catanzaro, Italy.

**Correspondence:** Angela Di Cesare (angdicesare@gmail.com)

Parasites & Vectors 2016, 10(Suppl 1):A45

Living in rural areas was identified as a risk factor for infection with *A. abstrusus* (p = 0.0001) and this accounted for most of the variation in regional prevalence. *Aelurostrongylus* was not detected in cats younger than 11 weeks and the prevalence in this age group was significantly lower than in older cats (p = 0.002). Based on these findings, lactogenic transmission seems unlikely, despite the fact that this route has been suggested for the closely related feline lungworm *Troglostrongylus brevior* [9]. The results of the present study demonstrated that *A. abstrusus* is endemic in Denmark. Therefore, this parasite should be considered an important differential diagnosis in any Danish cat displaying respiratory symptoms. The infection is especially relevant in outdoor cats living in rural areas. Other than rural origin, differences in regional prevalence may result from factors influencing the presence of intermediate and transport hosts, such as climate. However, socioeconomic differences between regions may also in part explain the differences in the current prevalence rates. With increased movement of pets, more extensive testing for *A. abstrusus* is warranted to monitor the distribution and prevalence of *A. abstrusus*.

**References**

1. Barutzki D. and Schaper R. 2013. Occurrence and regional distribution of *Aelurostrongylus abstrusus* in cats in Germany. Parasitol. Res. 112(2):855–861.
2. Traversa, D. and Cesare A. 2013. Feline lungworms: what a dilemma. Trends Parasitol. 29(10):423–430.
3. Payo-Puente, P., Botello-Dinis M, Uruena AMC, Payo-Puente M, Gonzalo-Orden JM and Rojo-Vazquez FA. 2008. Prevalence study of the lungworm *Aelurostrongylus abstrusus* in stray cats of Portugal. J Fe Med Surg. 10(3):242–246.
4. Traversa, D. and Guglielmini C. 2008. Feline *Aelurostrongylus abstrusus* and canine *Aelurostrongylus* a challenging diagnosis for two emerging venemous pneumonia infections. Vet. Parasitol. 157(3/4):163–174.
5. Di Cesare, A., Di Francesco G, Di Regalbono AF, Eleni C, De Liberato C, Marruchella G, Iorio R, Malatesta D, Romanucci MR, Bongiovanni L, Cassini R and Traversa D. 2015. Retrospective study on the occurrence of the feline lungworms *Aelurostrongylus abstrusus* and *Troglostrongylus* spp. in endemic areas of Italy. Vet. J. 203(2):233–238.
6. Genchi, M, Ferrari N, Fonti P, De Francesco L, Piazza C and Viglietti A. 2014. Relation between *Aelurostrongylus abstrusus* larvae excretion, respiratory and radiographic signs in naturally infected cats. Vet. Parasitol. 206(3/4):182–187.
7. Traversa, D., Lia PP, Iorio R, Boari A, Paradies P, Capelli G, Avolio S and Otranto D. 2008. Diagnosis and risk factors of *Aelurostrongylus abstrusus* (Nematoda, Strongylida) infection in cats from Italy. Vet. Parasitol. 153(1/2):182–186.
8. Olsen CS, Willesen JL, Piper CB and Mejer H. 2015. Occurrence of *Aelurostrongylus abstrusus* (Railliet, 1898) in Danish cats: a modified lung digestion method for isolating adult worms. Vet. Parasitol. 210(1/2):32–39.
9. Brianti E, Gaglii G, Napoli E, Falsone L, Giannetto S, Latrofa MS, Giannelli A, Dantas-Torres F and Otranto D. 2013. Evidence for direct transmission of the cat lungworm *Troglostrongylus brevior* (Strongylida: Crenosomatidae). Parasitology. 140(7):821–824.
morphological findings of the tail according to Georgi and Georgi (1991). In total 0.27% of faecal samples contained vital A. *vasorum* larvae (L1) and 2.66% of snails were infected with A. *vasorum*-larvae. Overall, these results show for the first time canine *A. vasorum* infections and also the presence of infected snails thereby proving the capability of this metastrongyloid nematode to adapt to new emerging intermediate hosts in Colombia. Thus, more research on epidemiology and biology of this neglected parasite and other closely related metastrongyloid nematodes with zoonotic potential are urgently needed in Colombia.

**A47**

Canine filarial and *A. vasorum* infections in an area of Central Italy (border Tuscany-Latium)

Fabio Macchioni¹, Marta Magi¹, Elisa Ulivieri¹, Francesca Gori¹, Manuela Schnyder²

¹Department of Veterinary Science, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy; ²Institute of Parasitology, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

Correspondence: Fabio Macchioni (fabio.macchioni@unipi.it)

Parasites & Vectors 2016, 10(Suppl 1):A47

Canine filarial infections are widespread throughout the world. New cases in dogs and in humans are occurring in many countries that were previously considered to be free or for which epidemiological data were not available [1]. *Angiostrongylus vasorum* is a cardiopulmonary parasite of wild and domestic canids. In Europe it is widespread in foxes, whereas in dogs it is “emergent” [2]. Both, filariae and *A. vasorum*, are increasingly reported in dogs in overlapping areas [3]. The aim of this work was to determine the occurrence of different species of filarial nematodes in dogs in an area of Central Italy at the border of two regions Tuscany-Latium, traditionally considered free and where epidemiological data in literature are lacking. Also the occurrence of *A. vasorum* was never investigated in this area. In the years 2015-2016 blood samples were collected from 100 dogs living in rural areas at the border between Tuscany and Latium, 50 sera from the province of Grosseto (Tuscany) and 50 from the province of Viterbo (Latium), respectively, and submitted to Knott’s test and ELISA for *Dirofilaria immitis* antigen detection (Dirocheck, Symbiotics®). Furthermore, 56 of these 100 dog samples were serologically tested for *A. vasorum* by ELISAs [4, 5]. Overall 54/100 dogs were positive for microfilariae. In Tuscany 17 out of 50 dogs (34%) were positive for dirofilariosis, i.e. 11 (22%) dogs were positive for *Dirofilaria immitis* and 6 (12%) for *Dirofilaria repens*. In Latium 10 out of 50 dogs (20%) were positive for dirofilariosis, of which 7 (14%) dogs were positive for *D. immitis*, 2 (4%) for *D. repens* and one dog (2%) had a concurrent infection with *D. immitis* and *D. repens*. Morphological identifications were confirmed by histochemical staining. Serological analysis for *A. vasorum* identified 3 cases (0.6%) originating from the Latium region, 2 of which were positive also for *D. immitis*. The results of this study highlight that canine filarial infections are expanding in previously considered free areas in Italy, as it is happening in many other countries. Single seropositive cases of *A. vasorum* anticipate the occurrence of this parasite in this area never investigated before. The presence of filarial infections in dogs suggests the need for prophylaxis in the study area, where it is actually not routinely performed.

References

1. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA. Human and animal dirofilariosis: the emergence of a zoonotic mosaic. Clin Microbiol Rev 2012; 25: S07–S44.
2. Taylor CS, Gato RG, Learmount J, Aziz NA., Montgomery C, Rose H, Wall R. Increased prevalence and geographic spread of the cardiopulmonary nematode *Angiostrongylus vasorum* in fox populations in Great Britain. Parasitology 2015; 142(09): 1190–1195.
3. Del Prete L, Maurelli MP, Pennacchio S, Bosco A, Musella V, Ciucu L, Rinaldi L. *Dirofilaria immitis* and *Angiostrongylus vasorum*: the contemporaneous detection in kennels. BMC Vet Res 2015; 11(1): 1.
4. Schnyder M, Tanner I, Webster P, Barutzki D, Deplazes P. An ELISA for sensitive and specific detection of circulating antigen of *Angiostrongylus vasorum* in serum samples of naturally and experimentally infected dogs. Vet Parasitol 2011; 179: 152–158.
5. Schucan A, Schnyder M, Tanner I, Barutzki D, Traversa D, Deplazes P. Detection of specific antibodies in dogs infected with *Angiostrongylus vasorum*. Vet Parasitol 2012; 185: 216–224.