Experimental assessment of vector competence of different mosquito species for Dirofilaria immitis

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MEETING ABSTRACTS

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TOPIC 1: Dirofilarioses (Humans, Mosquitoes)

A1
Human dirofilariosis in Europe: basic facts and retrospective review

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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


A2
Human dirofilariosis – morbidity, clinical presentation, and diagnosis

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As many as 3,545 cases of human dirofilariosis were recognized in Russia, Ukraine, and Belorusia 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 patients, 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
genitalia (3%) located parasite also visited a doctor in the first month of the disease. Female patients with breast location (3%) were consulted earlier and were undergone surgery in short time (in the first 2 weeks) mostly because the main diagnostic hypothesis was breast cancer. In the cases of extremities located parasitic nodule (hands – 9.4%, legs – 8.6%) only 31% and 36% of patients (accordingly) decided to be consulted by a doctor in the first month from the onset because they did not regard their condition as life-threatening. The next important issue is doctor information about dirofilariosis, his specialty and previous experience. Everything had great influence on timely and correct diagnosis or at least on inclusion of dirofilariosis in the list of diagnostic hypotheses. In the territories with sporadic morbidity only few doctors (7%) suspected dirofilariosis before surgery – they mostly diagnosed benign or malignant tumors (72%). There is a contrast with endemic territories where dirofilariosis was suspected by doctors in the much higher proportion of the patients (85%). Preliminary ultrasound and color Doppler examination of patients with dirofilariosis made great input in the diagnosis. The findings include hypoechoic encapsulated linear structures without internal blood vessels and sometimes (47%) with detectable movement of the parasite. Those findings allowed to exclude malignancies before surgery in all ultrasound examined patients. Medical community has to be better informed about dirofilariosis. Ultrasound should be a standard procedure in patients with subcutaneous nodules.

References

A4
An update on the current situation of Dirofilaria repens and Dirofilaria immitis in Austria
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Dirofilaria immitis and D. repens are filarioid helminths with domestic and wild canids as main hosts and mosquitoes as vectors. Both species are known to be zoonotic. Dirofilaria repens and D. immitis seem associated with climate change and a spread from historically endemic countries in Southern Europe to Central Europa was observed. Until very recently both species were known not to be endemic in Austria [1]. In Austria most cases of Dirofilaria spp. in humans and dogs are introduced. However, rarely infections with D. repens were discussed to be autochthonous. The introduction of D. repens to Austria was confirmed within a mosquito surveillance in Burgenland (Eastern Austria) for the first time in its vector [2,3,4]. We summarize not only introduced and possible autochthonous cases of Dirofilaria sp. in humans, dogs and vectors in Austria, but will also present data of mosquito screenings conducted after the first findings of D. repens in Anopheles algeriensis and the An. maculipennis-complex [3]. Moreover novel diagnostic tools for these filarioid helminths will be presented.

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References
A5

Experimental assessment of vector competence of different mosquito species for *Dirofilaria immitis*

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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*) in relation to local biotypes, under laboratory conditions.

Mosquitoes were reared at 24±2°C, with 85% humidity and fed with sugar solution. Six groups of female mosquitoes (30-50 individuals) were fed with blood containing *D. immitis* microfilariae (6,000 mf/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 humidity and fed with sugar solution. Six groups of female mosquitoes were dissected under a stereomicroscope on days 2, 4, 7, 10, 14 post inoculation (p.i.), to observe the developmental stages of microfilariae (L1, L2, L3) that 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 *Cx. pipiens* biotype molestus and *Ae. geniculatus* and further studies will be necessary with these species.

A6

Advances in adulticide treatment in canine heartworm disease (*Dirofilaria immitis*): Macroyclic lactones and doxycycline

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Currently, the only registered drug for adulticide therapy in dogs with heartworm disease (HWD, *Dirofilaria immitis*) is melarsomine dihydrochloride (Immiticide®, Merial). The American Heartworm Society (AHS), based on results from several studies [1, 2], currently recommends that, in cases where arsenical therapy is not possible or is contraindicated, a monthly heartworm preventive along with doxycycline for 4-week period might be considered. There is no published data on the use of moxidectin in combination with doxycycline. Preliminary results of an on-going study* show that moxidectin, the only macrocyclic lactone (ML) registered as a microfilaricide, is also adulticidal when combined with doxycycline. Interestingly, a similar combination therapy has been shown to be highly effective against human body lice, an ectoparasite that has been shown to develop resistance to MLs and which also harbours bacterial endosymbionts [3]. It is not yet known if the efficacy of antibiotics and MLs is due to pharmacokinetic or pharmacodynamic synergism. It has been shown that compounds including antibiotics can increase intracellular concentrations of MLs and that MLs can inhibit cell detoxification mechanisms, thus increasing intracellular concentrations of drugs, including antibiotics [4]. A recent study has shown, however, that serum levels of doxycycline in dogs treated with the combination protocol were not statistically different compared to dogs treated with doxycycline alone [5]. It would be of interest, and a research priority, to elucidate the nature of this synergy.

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References


Cortisol as indicator of stress in heartworm infection in dogs
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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 microfilaric and 20 were amicrofilaric; 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 microfilaric 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
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

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**References**


**A10**

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

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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 (suspect 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 erythroemia, lumphopecia, 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
A11
A12

A11
Awareness and strategies about canine heartworm (Dirofilaria immitis) infection in private practices in Greece: preliminary results of an ongoing questionnaire survey

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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 routinely implemented strategies on diagnosis, treatment and prevention. Until today, 134 questionnaires have been completed; 51.5% from the Northern and Central Greece (NCG) and 48.5% from the rest of the country (RC, continental and insular). The percentage of veterinarians reporting that they see at least one HW case per month, trimester, semester and year was 22.3%, 18.5%, 16.1% and 15.3%, respectively, while no cases of HW was reported from 26.1% of the participants. The criteria for suggesting prevention included the geographical area where the animal lived (88.8%), its lifestyle (72%), breed (13%), and the owner’s compliance (41.1%). Most of the veterinarians (61%) suggest prevention measures all year round, while some (35.5%) only during the warm season of the year. Regarding treatment, 50% of the veterinarians consider as first choice the protocol endorsed by the American Heartworm Society (AHS) and the European Scientific Counsel Companion Animal Parasites (ESCCAP), while 16% apply a “slow kill” protocol. For the prevention of pulmonary thromboembolism 84% of the veterinarians suggest strict activity restriction, 67% administer prednisolone while 28% use aspirin.

There is a recorded difference of awareness of HW between the NCG and RC veterinarians that could be attributed to the indication of higher prevalence of HW in NCG. Indeed, only 10.3% of the NCG veterinarians report absence of HW in their area, while the corresponding percentage in RC is 40.9%. In NCG, 73.5% of the veterinarians suggest appropriate preventive measures (endorsed by the AHS and the ESCCAP) while in RC only 42.4% suggest such measures. Moreover, 85.3% of NCG veterinarians advise HW prevention for all dogs, while the respective percentage in RC is only 12.1%. These results suggest that although the majority of small animal practitioners in Greece appear to generally comply with the updated guidelines on the prevention and treatment of HW, the geographical area-based perceived risk for HW substantially affects the preventive strategy implemented.

References

A12
Atypical case of subcutaneous filariasis in a cat: do we expect Dirofilaria immitis there?

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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 excided and examined. Histology showed in the subcutis the presence of a nodular lesion characterized by a severe inflammatory infiltrated 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 polymyarian-coelomyarian musculature. Microscopic features were compatible with D. immitis morphology. [2] After extraction from the paraffin block, DNA of 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 antige- numia 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
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 melarsamine 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 doxycycline (10 mg/kg every third month) and prophylactic dose of ivermectin (10 mcg/kg every 15 days). Therapy was given 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


**TOPIC 4: Dirofilarioses (Veterinary Medicine/Epidemiology/Tropical Parasitology)**

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* microfilaria, 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® ADX™ test and PCR amplifying the fragment of COI gene of filarial nematodes [5]. Only *D. repens* was 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 Zlín 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 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.

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References

5. Cissiraghi 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 mosquitos 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. Informing and advising 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 mosquitos 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 melarso-mine injections. Informing and advising animal welfare associations and dog owners as well as identification of imported and infected ani-

mals and the rapid onset of a safe therapy is the only way to delay or even avoid heartworm disease becoming endemic in Austria.

References


of *D. immitis* in red foxes from Portugal and to evaluate their potential 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, Vila, Diana do 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, showing 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 Portuguese fox population to allow a better management of its vector-borne infections and diseases, in line with the ‘One Health’ concept.

**Funding**

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**References**

Dirofilaria repens is not a life-threatening parasite, however it is one of the major differential diagnosis that must be done when blood microfilariae are detected in a dog. This was the case in a 20 month-old Tosa dog that came to the surgery consultation of the Small Animals Veterinary Hospital of Alfort, France in February 2016. The dog had a subcutaneous skin nodule on the head. The medical imaging examination, performed the day of the consultation, showed a well-defined nodule of 1.5 cm of large and 5 mm of depth, with several hyperchoic lines inside. The nodule was punctured with a small needle and polynuclear granulocytes as well as microfilariae were detected, after staining. Blood analysis revealed the presence of microfilariae both in the smear and in the sediment following the Knott technique (mean 176 larvae/ml). After the surgical removal of the skin nodule, one nematode of 8 cm of length was found inside, identified by means of PCR (ITS) as Dirofilaria repens. Blood analysis performed 6 weeks later, in the absence of any treatment, demonstrated a decrease of microfilaraemia of 22%. The dog was imported from South of Romania, at the age of 3 months, and since then, the dog never left Ile de France region. Since both in Romania and in Ile de France region, several cases of subcutaneous dirofilariosis have been described so far in dogs and cats, it is not possible to identify with certainty the place where the initial contamination occurred. However, the region of south Romania, where the dog was born is highly endemic for mosquitoes.

A19

Serological survey of Dirofilaria in humans from Romania and Republic of Moldova

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Dirofilariosis is an emerging zoonotic infection caused by the filarial nematodes of dogs Dirofilaria repens and Dirofilaria immitis [1]. The high prevalence of both Dirofilaria species in dogs in Romania represent a constant threat for animal and public health. However, only few cases of human infections by D. repens have been reported from various regions of Romania so far. In the present study a serological screening was performed on a cohort of patients from Romania (n = 187) and Republic of Moldova (n = 263) for a total of 450 patients (166 male and 284 females; aged 7 to 78 years). Sera samples were collected from December 2015 to March 2016 and analyzed by a non-commercial IgG-ELISA for the detection of IgG anti-D. immitis and anti-D. repens. Of 187 patients from Romania 13 (6.9%) were positive for D. immitis, and only one patient reacted against both antigens of D. immitis and D. repens. Of 263 patients from Republic Moldova, 36 (13.6%) were positive for D. immitis and three (1.4%) patients recognized both antigens. Only one patient was found positive for IgG-anti D. repens. Moreover the results from the present study were confirmed by Western blot analysis, which gives even greater support to these results. Our results confirmed the public health significance and zoonotic impact of Dirofilaria infections in Romania and the Republic of Moldova. Considering that the main reservoir is represented by the microfilaricem dogs and the infections transmitted by mosquitoes, entomological surveillance and monitoring of dogs are needed in both countries, in order to define the risk areas of infection.

References


A20

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

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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 Dipyllidium 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). Titters 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 pahangi 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

Human dirofilariasis 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 subconjunctival and periocular infestation. Aim of this paper is to report new *D. repens* infections diagnosed in our country, and to address attention that this benign infection may have serious clinical course. We present three cases of human dirofilariasis, two autochthonous and one imported, diagnosed from February 2015 till April 2016. All patients were adults, one male and two females, two with subcutaneous infection 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 performed and white thread like 8 cm long mass was extracted and amoxicillin/clavulanic acid (100 mg twice/day for 15 days) was prescribed. Diagnosis of a *D. repens* infection was made in pathohistological 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* infection was imported from Tivat, Montenegro in a 22 year old female. The infection was manifested as two weeks increasing subcutaneous nodule (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 diagnosed, incision was performed and 9 cm worm was extracted.

In the previous two cases *D. repens* was identified according to morphological features in histological sections. All patients provided agreement for participation in this study.

References


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 potential 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 Belarus; *Cx. theleri* 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. daceae*, *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 differs of the feeding patterns of each specie. 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
The first published research on *Dirofilaria immitis* (*D. immitis*) infections in Serbia was in the 1990s, when the first cases were determined 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 dirofilariosis 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 regular health check-up in dogs is provided by the veterinary service. Herein we report a very high prevalence of *D. immitis* infections 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* infections which were diagnosed at clinical examinations (dogs presented cough and weakness during the regular everyday activities), snap test (SNAP 4DX Idexx) and by Knott test for detection of microfilariae 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 Knott testing. After therapeutic procedure, microfilariae were not detected in blood of all cured dogs. Therapy with Ivermectin and Knott test were repeated for the next 6 months and there were no parasitological positive findings of dirofilariosis. In addition, eight months after the first therapy was given to all of the dogs, parasitological and clinical examinations showed that all dogs were without clinical symptoms and using Knott test microfilariae were not found in blood of examined dogs.

### A25

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

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Dirofilariosis 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 alveolar and interstitial pattern in both animal and public health [1]. Greece is a typical Mediterranean country with reported cases of Dirofilaria-infected animals [2] and has favourable climatic conditions for mosquitoes, including new invasive species. The aim of this study was to investigate the presence of *Dirofilaria* spp. in clinically healthy dogs and to create a prevalence map including all geographic parts of the country. Blood samples were collected from a total of 276 dogs. They were animals of different breeds, both indoor and outdoor and used for different purposes (hunting, guarding, pets, shepherds, stray etc.). All samples were tested with the SNAP® 4DX® Plus Test to detect *Dirofilaria immitis* antigen. In addition, samples were examined by Knott test to identify microfilariae of *D. immitis* and *D. repens*. Additional data were collected in order to identify potential risk factors. Thirty-two *D. immitis* antigen positive samples (11.6%) were detected by serology and 19 of 32 were amicrofilariaemic. *D. repens* microfilariae were identified in 4 (1.4%) dogs. Infected dogs were originating significantly more from Northern than Southern parts of Greece (*p < 0.01*). Also, dogs at higher risk were the ones spending more time or activity outside the house (i.e. hunting) and with minimum preventive antiparasitic administration. These 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.

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**References**


### A26

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

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Thoracic radiography is a very important diagnostic procedure for establishing a diagnosis of the heartworm disease (HWD). It enables 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 determination 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 disease were measured. Both recumbent lateral (LL) and dorsoventral (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 results of wet blood smears, modified Knott test [1] and heartworm antigen test. Radiographs of each dog were assessed subjectively (vascular, alveolar and interstitial pattern and right-sided cardiomegaly) [2] and objectively (VHS) [3, 4]. A stage of heartworm disease was determined for each dog. Results were statistically analyzed and presented as percentages (qualitative variables) and mean±standard deviation (SD) (quantitative variables). In this descriptive 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 assessment 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 pattern. 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 caudal lobar artery 0.96 ± 0.42v. Further studies are needed to compare these results with results of dogs without HWD in order to define the most important changes that could be used as a diagnostic or prognostic tool. Objectivity in the assessment of the radiographs of dogs with HWD is possible to achieve by scoring the findings and using objective radiographic measurement.

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References

A27
Occurrence and taxonomical classification of microfilariae in blood samples from canine blood donors localized in south-eastern Poland
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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 microfilariasis among canine blood donors. The survey was carried out in the second half of year – between May and December 2013 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, Acanthocheloneema 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 infection and possible immune reactions of the host (blood recipient) indicate that screening tests for dirofilariosis are essential.

References

A28
Filaroid helminths in mosquitoes from the Danube Delta/Romania and the analysis of these vectors for potential vector competence
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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. 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. Mosquitoes were segregated into head/thorax and abdomen prior to DNA extraction. Each thorax/head and abdomen were screened for the presence of filarial DNA separately. All positive PCR products were further analysed by sequencing.

Mosquitoes were sampled within the training school of WGI under the frame of EurNegVec COST Action TD1303. Parts of this study were funded by the ERA-Net BiodivERsA, with the national funders FWF I-1437, ANR-13-EBID-0007-01 and DFG BiodivERsA KL 2087/6-1 as part of the 2012-13 BiodivERsA call for research proposals.

References

4TH BAYER ANGIOSTRONGYLOSIS FORUM 2016

A29
Angiostrongylus vasorum – what’s new?
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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 development 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 cardiac 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 neutrophil 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.

### A30

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

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**Parasites & Vectors 2016, 10(Suppl 1):A30**

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 Europe. 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.

### A31

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

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**Parasites & Vectors 2016, 10(Suppl 1):A31**

*Angiostrongylus vasorum*, the French heartworm, is a parasite of dogs described in several parts of the world, including continental Europe and the British Isles [1]. Regarding parasite occurrence in Scandinavia, endemic foci are widely present in Denmark and recently the parasite has been found in foxes in Norway [2]. In Sweden the parasite was first identified in 2003 on the island of Sydkoster (Västra Götaland County) [3]. Since then Swedish sporadic endemic cases of *A. vasorum* were diagnosed through positive canine faecal samples every year since 2011. A progressively increasing number of faecal samples has been submitted to SVA (National Veterinary Institute, Uppsala), however the prevalence in dogs appears to be quite low. A large-scale collection of canine serum samples was planned in order to identify the prevalence and distribution of *A. vasorum* in Sweden using more sensitive diagnostic methods, i.e. serological methods able to detect parasite antigens and antibodies developed against the parasite. In this first large scale survey, 3886 sera from pet dogs were collected from the Clinical Chemistry Laboratory of the University Animal Hospital (UDS-5LU, Uppsala) as well as from SVA and 3309 (85% of 3886) have been tested until now by an ELISA for the detection of circulating antigen of *A. vasorum* and by a separate ELISA detecting specific antibodies against the parasite. Among the analysed samples a total of 0.39% (n = 13, 95% Confidence Intervals, CI: 0.21-0.67%) of the animals were positive in both ELISAs, while 0.70% (n = 12, CI: 0.44-1.04%) of the tested dogs were antigen-positive only and 1.48% (n = 49, CI: 1.48-1.10%) were positive for specific antibodies only. These preliminary results confirm that *A. vasorum* is established in Sweden with a prevalence comparable to other European countries. Definitive results from ongoing analyses will provide a deeper insight on the dissemination of the parasite over the country.

**References**

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 antimicrobial therapy, this leads to the potential for under-diagnosis of lungworms. A reliance in clinical practice on fecal flotation for detection of parasites is frequent. Diagnosis is challenging due to poor detection sensitivity and 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 hirshi/Oslerus osleri (0.3%; 1/317) and Aeukrostrongylus abstrusus (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 Eucoelsus 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) detection 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.

A33
Baermann fecal examination survey of dogs showing signs of respiratory disease in Ontario, Canada
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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 detection of parasites, this leads to the potential for under-diagnosis of lungworms. A further complication is the sporadic fecal larval shedding patterns typical of metastastrongylid 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 zinc sulphate centrifugal flotation from October 2014 to May 2016. Afebrile 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 hirshi/Oslerus osleri (0.3%; 1/317) and Aeukrostrongylus abstrusus (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 Eucoelsus 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) detection 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.
A35

A coprological and serological survey on Angiostrongylus vasorum in Southern Belgium

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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™) were 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. Forty-six dogs out of 979 (4.7%) had A. vasorum circulating antigen. However, there was a marked difference between the two populations (3.6 and 8.6% in control and symptomatic dogs respectively). Stools were obtained from 47 dogs (25 and 22 in control and symptomatic dogs respectively). Interest-ingly larvae of Crenosoma vulpis were detected in 1 control and 8 symptomatic dogs respectively. In the latter group one dog was found seropositive for A. vasorum but only C. vulpis larvae were found via the Baermann technique. All seropositive and symptomatic dogs (n = 19) exhibited cardio-pulmonary symptoms. In conclusion this seroepidemiological study demonstrated a fairly high seroprevalence in Southern Belgium for A. vasorum. The Angio detect™ IDEXX was found to be highly suitable in this context as the sampling, pres-ervation and examination of stools were difficult and somewhat un-reliable in the field. However, coproscopy remains a useful tool in dogs infected for less than 9 weeks and for the identification of other canine lung nematodes such as Crenosoma vulpis.

References

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

<table>
<thead>
<tr>
<th></th>
<th>Control dogs (n=757) (%)</th>
<th>Symptomatic dogs (n=222) (%)</th>
<th>Total (n=979) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angio detect™ IDEXX +</td>
<td>27 (3.6)</td>
<td>19 (8.6)</td>
<td>46 (4.7)</td>
</tr>
<tr>
<td>Angio detect™ IDEXX + and Baermann +</td>
<td>1 L1 A. vasorum</td>
<td>7 (1.0)</td>
<td>6 (2.7)</td>
</tr>
<tr>
<td>Angio detect™ IDEXX + and Baermann +</td>
<td>1 L1 A. vasorum and C. vulpis</td>
<td>1 (0.45)</td>
<td>1 (0.45)</td>
</tr>
<tr>
<td>Angio detect™ IDEXX + and Baermann not performed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angio detect™ IDEXX – and Baermann +</td>
<td>17 8 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angio detect™ IDEXX – and Baermann +</td>
<td>3 2 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angio detect™ IDEXX –</td>
<td>3 2 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A36

Risk factors for natural infection with Angiostrongylus vasorum in dogs: 100 cases (2003-2009)

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Canine angiostrongylosis is a snail-borne parasitic infection caused by the nematode Angiostrongylus vasorum. The nematode has a complex life cycle, potentially involving an array of intermediate, paratenic and definitive hosts. The global geographical boundaries of infection are spreading to encompass areas where infection was previously uncommon, thus presenting a growing threat to the canid population. Clinical signs of A. vasorum infec-tion in dogs are serious and can potentially lead to death. This retrospective study was conducted to evaluate trends in demographic factors and clinical presentation of 100 dogs with A. vasorum. Variables analyzed included dog age, breed, gender and frequency of clinical signs. A significant relationship was detected between young age and A. vasorum infection, supporting the hypothesis that age-related differences exist in response to A. vasorum infection. Gender was not identified as a significant risk factor associated with A. vasorum infection in dogs. The breeds that have the highest prevalence of angiostrongylosis were Cocker Spaniels and Labradors, with 13% and 12% of dogs re-spectively. Significant association was made between A. vasorum infection and dogs presenting with cough, coagulopathy, vomit-ing/diarrhoea and/or lethargy (p < 0.05). Taking account of these classical clinical presentations gundogs are highly likely to present with all signs, whereas terriers are presented less often with a coagulopathy (9%) than with the other signs, and hounds are less often with a cough (2%). These findings are important because they provide clues regarding the risk of infection to an individual dog, facilitate improved recognition of infection based upon clinical presentation, and should allow implementation of preventative strategies to combat infection.
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 distribution of lungworms 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 aerophilus and Crenosoma vulpis were identified. C. aerophilus 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. aerophilus 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 metastrongyloid species were very limited. We therefore present reliable 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 metastrongyloid species were very limited. We therefore present reliable and specific detection of circulating antigen of Angiostrongylus vasorum in serum samples of naturally and experimentally infected dogs. Veterinary Parasitology, 2011; 179(1-3): 152–158.

References

A38 Gamropriod shedding of third-stage larvae after infection of metastrongyloid lungworms
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Felids and canids acquire infection of metastrongyloid nematode parasites (AeluropDstrohbus straburus, Angiostrongylus vasorum, Crenosoma vulpis, Olsner rostratus, Troglostrongylus spp.) by the ingestion of infective third-stage larvae (L3) in the tissues of gastropod intermediate hosts (IMH) and in some species also paratenic hosts. Speculation on potential exposure due to L3 shed into the environment has arisen due to reports of L3 released by laboratory aquatic snails experimentally infected with A. abstrusus, A. vasorum and Troglostrongylus brevior. Spontaneous shedding has not been reported for these species in the terrestrial gastropod natural IMH (slugs, land snails). First-stage larvae of A. abstrusus, A. vasorum and C. vulpis were each placed on lettuce (400 – 1445 L1/slug) and fed to laboratory raised Limax maximus in separate exposure groups of 12-42 slugs. In addition, a mixture of O. rostratus-T. wilsoni (90%/10%) and T. wilsoni-O. rostratus (95%/5%) L1 recovered from a co-infected bobcat were used to infect 30 and 22 L. maximus (1600 L1/slug), respectively. Slug feces was examined for L3 (2x/week) using a modified Baermann method. All surviving slugs were digested for individual L3 counts at the termination of the studies (105 – 210 days PI). Shedding of L3 was detected in all groups beginning 20-30 days PI and continued from 55 to 202 days PI. No L3 were detected from unexposed control group slugs. Weekly shedding levels for the various infection groups ranged from 0 – 3.273 L3/slug. The percentage of L3 shed ranged from 1.3% (A. vasorum) to 2.77% (C. vulpis) of the total recovered (= shed L3-digest L3) from each exposure. Mortality rates in infected slugs ranged from 0% (A. abstrusus) to 65% (Crenosoma vulpis). Based on identification using morphology, L3 of both T. wilsoni and O. rostratus were shed in both of the mixture-exposed groups. Longevity in the environment was tested by placing L3 of A. vasorum and T. wilsoni-O. rostratus on lettuce. Actively motile L3 were recovered from the lettuce for up to 16 days post-deposit. Detection of spontaneous shedding in all 5 parasite species indicates that spontaneous shedding of L3 into the environment is likely a general characteristic of the metastrongyloids. The spontaneous shedding and prolonged survival of L3 indicates that exposure through environmental contamination likely plays a role in infection transmission with these parasite species.

A39 Angiostrongylus chobaudi: first description of the diagnostic stage and confirmation of European wildcat as definitive host
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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 wild-cats (*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 x 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 extend 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**

Angiostrongylus chabaudi and A. daskalovi in wild carnivores from Romania

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Angiostrongylus chabaudi is a rare feline cardio-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 (cox1) 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.

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
be difficult, because their identification is particular based on the tail shape [2]. Unlike the Baermann method, flotation-based techniques are easy to perform, 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 exposure 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 (S.G). Fresh fecal sample (6 g) from natural infected with A. abstrusus 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 floatation solutions respectively: 33% ZnSO4 (SG 1.18), saturated NaCl (SG 1.20), commercially available NaNO3 - Fecasol (SG 1.20), saturated NaCl and sacharose (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. abstrusus were found in all floatations. The biggest recovery rates 3.2 and 3.1 were achieved using floatation solutions with the highest specific gravities (saturated solutions of: NaCl/sacharose 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

Table 2 A Comparison of various flotation fluids in A. abstrusus larvae (L1) detection

<table>
<thead>
<tr>
<th>flotation solutions S.G</th>
<th>recovery rates for LPG 2800</th>
<th>number of larvae in flotation</th>
<th>identifiable % of identifiable average length of larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>33% ZnSO4 (SG 1.18)</td>
<td>0.5</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>saturated NaCl (SG 1.20)</td>
<td>1.1</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>commercially available NaNO3 - Fecasol (SG 1.20)</td>
<td>1.0</td>
<td>28</td>
<td>20</td>
</tr>
<tr>
<td>saturated NaCl and sacharose (SG 1.25)</td>
<td>3.2</td>
<td>90</td>
<td>28</td>
</tr>
</tbody>
</table>

A43 A ten-year retrospective study of angiostrongylosis at Alfort Veterinary School, Ile de France
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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 7 last 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
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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%.
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 [10]. 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

A46
First report of Angiostrongylus vasorum infections in dogs as well as in the neozoan intermediate host (Achatina fulica) in Medellin, Colombia
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Angiostrongylus vasorum is considered as one of the most pathogenic species of the cardipulmonary system of wild and domestic canids worldwide. As such, in 1961 this metaostronygloid parasite was detected in South America in crab-eating foxes (Cercdoycon 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 neozoan intermediate hosts by analysing dog faecal samples collected in public parks in Medellin city and from collected neozoan terrestrial snails (Achatina fulica). In total 364 faecal samples were collected from February to April 2016, analysed therewith by Beamann 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...
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)
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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, Synbiosis®). 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 histocchemical 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