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Toxocara2012

***Toxo**cara2012*

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Abstract Booklet

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Introduction

This booklet contains submitted abstracts from the invited speakers and poster presenters at the ESCCAP ***Toxo****cara2012* conference, organised by the European Scientific Council for Companion Animal Parasites (ESCCAP®). The conference, which was held in Budapest, Hungary, between 3rd and 5th October 2012, examined medical, veterinary and biological aspects of *Toxocara*. The meeting served as a rare opportunity for those interested in this parasite from a diversity of perspectives to meet and exchange information and ideas. The meeting attracted over one hundred participants who listened to ten invited speaker presentations. Internationally recognised experts in the field delivered presentations on topics covering diagnosis and disease in humans; veterinary aspects of infection, with a particular focus on how to determine which animals contribute the most to environmental contamination; and biological aspects, including immunity and infection in the paratenic host. The presented papers can be viewed on the ESCCAP channel of the KeySkill learning platform at www.keyskill.com.

Toxocara spp. have fascinating life cycles with mainly carnivores as definitive hosts including domestic dogs and cats, wild canids and felids. Many other mammals and birds are acting as paratenic hosts and reservoirs of the parasite. Several modes of parasite transmission, including the highly efficient intrauterine transmission employed by *Toxocara canis* in canids, makes this parasite one of the most frequent parasite infections in dogs in Europe. These unique and fascinating biological features coupled with the fact that humans can be accidental and aberrant hosts of *Toxocara* and related species, with potentially serious clinical outcomes such as ocular or neurological toxocarosis, visceral larva migrans and allergic related diseases, means that the control of this complex disease requires an interdisciplinary effort and a unified approach across both human and veterinary medicine, reflecting the One Health approach. In the near future, the omics technologies, improved diagnostics, and animal and immunological models, all of which were reviewed by the speakers at this 2012 Budapest meeting, could provide substantial opportunities for further exploring fundamental questions of parasite biology and pathogenesis.

The meeting and the subsequent special edition of **Veterinary Parasitology** would not have been possible without the generous support provided by the meeting sponsors: Bayer, Novartis and MSD Animal Health. Our thanks go to our sponsors, to the ESCCAP Secretariat for their excellent organisation of the meeting and to all of the contributors to the meeting. We sincerely hope that the meeting provided the catalyst for renewed enthusiasm for research into *Toxocara* spp. and toxocarosis, as well as subsequent meetings and new networks.

MA Fisher, P Deplazes

Guest Speaker Paper Abstracts

Lung histopathology, radiography, high-resolution CT, and bronchio-alveolar lavage cytology are altered by *Toxocara cati* infection in cats and is independent of development of adult intestinal parasites

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Abstract

This study presents clinical findings after oral ingestion of *Toxocara cati* eggs which resulted in rapid pulmonary lung migration and parenchymal disease, noted on clinically relevant diagnostic methods. Further, the study investigated the efficacy of pre-infection applications of preventative medication on larval migration through the lungs. A third aim of the study was to determine if adult cats infected with *T. cati* developed lung disease. Cats in infected groups were administered five oral doses of L3 *T. cati* larvae. Four-month-old specific pathogen free (SPF) kittens were divided into three groups (six per group): an infected untreated group, an uninfected untreated control group, and an infected treated group (topical moxidectin and imidacloprid, Advantage Multi for Cats, Bayer Healthcare LLC). Six 2- to 3-year-old adult multiparous female SPF cats were an infected untreated adult group. The cats were evaluated by serial CBCs, bronchial-alveolar lavage (BAL), faecal examinations, thoracic radiographs, and thoracic CT scans and were euthanised 65 days after the initial infection.

Adult *T. cati* were recovered in infected untreated kittens (5/6) and infected untreated adults (5/6) in numbers consistent with natural infections. Eggs were identified in the faeces of most but not all cats with adult worm infections. No adult worms were identified in the uninfected controls or the infected treated group. All cats in the infected groups, including treated cats and untreated cats without adult worms, had lung pathology based on evaluation of radiography, CT scans, and histopathology.

The infected cats demonstrated a transient peripheral eosinophilia and marked eosinophilic BAL cytology, but normal bronchial reactivity based on *in vivo* CT and *in vitro* ring studies. Lung lesions initially identified by CT on day 11 were progressive. Thoracic radiographs in infected

cats had a diffuse bronchial-interstitial pattern and enlarged pulmonary arteries. Pulmonary arterial, bronchial, and interstitial disease was prominent in histological findings. Infected treated cats had a subtle attenuation but not prevention of lung disease compared to infected cats. Significant lung disease in kittens and adult cats is associated with the early arrival of *T. cati* larvae in the lungs and is independent of the development of adult worms in the intestine. These data suggest that while the medical prevention of the development of adult parasites after oral exposure to *T. cati* is obviously beneficial, this practice even with good client compliance will not prevent the development of lung disease which can alter clinical diagnostic methods.

Keywords: *Toxocara cati*, pulmonary fibrosis, high resolution CT, larval migration

Factors affecting disease manifestation of toxocarosis in humans: genetics and environment

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Abstract

Toxocara canis is regarded as the main cause of human toxocarosis but the relative contribution of *T. cati* is probably underestimated; serological and other diagnostic methods used in most studies of this zoonotic disease do not distinguish between the two parasites. The definitive hosts for *T. canis* are canidae. Pups generally have higher infection rates than adult animals and are a major source of eggs in the environment. Humans usually acquire *T. canis* infection by accidental ingestion of embryonated eggs or encapsulated larvae from the environment or contaminated food, such infections may lead to visceral larva migrans (VLM), ocular larva migrans (OLM) or covert toxocarosis (CT). Although a mixed Th1- and Th2-mediated immunological response, particularly with high levels of IgE and eosinophilia is observed, the underlying mechanisms of molecular and immunopathogenesis for the development of the symptomatic syndromes of VLM, OLM, or of asymptomatic CT are largely unclear. Studies have indicated that immunological defences against various infectious diseases may be highly influenced by complex interactions of environmental and host genetic factors e.g. MHC class I and II, also known as human leukocyte antigen (HLA). *Toxocara* spp. infections are associated with a polarized $CD4^+$ Th2 response with high IgE levels and eosinophilia, mediated mainly by HLA class II molecules. Associations have been made between HLA class II and pathological severity and host genetic effects on exposure to infection. Recent research suggests $Foxp3^+CD4^+CD25^+$- expressing T regulatory (Treg) cells play a role in the regulation of the immunopathology of granulomas in experimental toxocaral granulomatous hepatitis and in enhanced expression of TGF-β1, which is an important factor for the local survival and function of Treg observed during *T. canis* invasion in the mouse small intestine, liver, muscle, and brain. Since the potential susceptibility loci HLA class II molecules, are considered involved in the regulation of a Th2-dominant immunity which is highly controlled by $FoxP3^+$ $CD4^+CD25^+$ Treg cells by stimulation through TGF-β1, which thus provides

a beneficial environment to *T. canis* larvae but severe injuries to local organs. However, TGF-β1 variant Leu10Pro known to be involved in disease severity warrants further elucidation as this too may have a role in the severity of human toxocarosis. Exploration of TGF-β1 polymorphism, $Foxp3^{+}$ $CD4^{+}CD25^{+}$ Treg cells, and MHC polymorphisms may allow insight into the contribution made by environmental and genetic factors in influencing disease syndrome type and severity in humans with toxocarosis.

Keywords: Human toxocarosis; HLA; TGF-β1; $FoxP3^{+}$ $CD4^{+}CD25^{+}$ Treg cells

Laboratory diagnosis of human toxocarosis

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Abstract

Toxocarosis is a helminth zoonosis caused by infection with the larvae of *Toxocara* spp. ascarid worms. Only two species, *Toxocara canis* and *Toxocara cati,* are recognised as causative agents of human disease. The best choice for serodiagnosis of the generalised forms of toxocarosis, VLM or covert toxocarosis, relies upon the initial use of TES-ELISA, after which any positive result should subsequently be tested by Western blotting (WB). Covert toxocarosis is mostly a benign infection, so a large majority of infected subjects are asymptomatic or have very few symptoms and therefore go undiagnosed. In this form, this helminthosis is often self-limiting, leaving residual specific antibodies. A positive serodiagnosis caused by residual antibodies that do not have any diagnostic significance can be associated with any infectious or non-infectious disease. If separated from the ongoing clinical and laboratory context, such a positive result has no diagnostic value and should be only taken into account after the possible aetiologies of any observed syndromes have been ruled out. Unlike the methods used for the immunodiagnosis of bacterial, viral or protozoal (toxoplasmosis) infections, it is not possible with toxocarosis to assess the age of the presence of specific IgG using the levels of specific IgM because IgM antibodies can be found throughout the course of helminthosis. The detection of other classes of immunoglobulins, namely IgE and IgA, the subclasses, namely IgG4 or circulating Ag was proven to be unable to discriminate between active and self-cured generalised toxocaral infections. Currently, the diagnosis of an active covert toxocarosis relies upon indirect arguments, e.g., the presence of otherwise unexplained symptoms along with blood eosinophilia and/or elevated levels of eosinophil cationic protein (ECP). This situation is far from ideal and more research should be carried out to solve this difficult problem.

Keywords: Human toxocarosis, immunodiagnosis, active infection, diagnostic strategy

A perfect time to harness advanced molecular technologies to explore the fundamental biology of *Toxocara* spp.

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Abstract

Toxocarosis is of major canine health and socio-economic importance worldwide. Although many studies have given insights into toxocarosis, to date, there has been limited exploration of the molecular biology, biochemistry, genetics, epidemiology and ecology of *Toxocara* spp. as well as parasite-host interactions using '–omic' technologies. The present article gives a background on *Toxocara* species and toxocarosis, describes molecular tools for specific identification and genetic analysis, and provides a prospective view of the benefits that advanced molecular technologies will have toward better understanding the parasites and disease. Tackling key biological questions employing a 'systems biology' approach should lead to new and improved strategies for the treatment, diagnosis and control of toxocarosis.

Keywords: toxocarosis, *Toxocara canis*, *Toxocara cati*, ascaridoids, molecular biology, parasite-host interactions, disease

Toxocara canis: molecular basis of immune recognition and evasion

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Abstract

Toxocara canis has extraordinary abilities to survive for many years in the tissues of diverse vertebrate species, as well as to develop to maturity in the intestinal tract of its definitive canid host. Human disease is caused by larval stages invading musculature, brain and the eye, and immune mechanisms appear to be ineffective at eliminating the infection. Survival of *T. canis* larvae can be attributed to two molecular strategies evolved by the parasite. Firstly, it releases quantities of 'excretory-secretory' products which include lectins, mucins and enzymes that interact with and modulate host immunity. For example, one lectin (CTL-1) is very similar to mammalian lectins, required for tissue inflammation, suggesting that *T. canis* may interfere with leukocyte extravasation into infected sites. The second strategy is the elaboration of a specialised mucin-rich surface coat; this is loosely attached to the parasite epicuticle in a fashion that permits rapid escape when host antibodies and cells adhere, resulting in an inflammatory reaction around a newly vacated focus. The mucins have been characterised as bearing multiple glycan side-chains, consisting of a blood-group-like trisacccharide with one or two O-methylation modifications. Both the lectins and these trisaccharides are targeted by host antibodies, with anti-lectin antibodies showing particular diagnostic promise. Antibodies to the non-methylated trisaccharide appear to be *T. canis*-specific, as this epitope is not found in the closely-related *T. cati*, but all other antigenic determinants are very similar between the two species, which will be important in designing new and more accurate diagnostic tests. Further tools to control toxocarosis could also arise from understanding the molecular cues and steps involved in larval development. *In vitro*-cultivated larvae express high levels of four mRNAs that are translationally silenced as the proteins they encode are not detectable in cultured larvae. However, the four proteins appear to be produced once the parasite has entered the mammalian host, as they are recognised by specific antibodies in infected patients. Elucidating the function of these genes, or analysing if micro-RNA translational silencing

suppresses production of the proteins, may point towards new drug targets for tissue-phase parasites in humans.

Keywords: antibodies, diagnosis, larva migrans, mucins, surface coat

Quantifying sources of environmental contamination with *Toxocara* spp. eggs

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Abstract

A rich body of work has reported levels of infection with *Toxocara* species in definitive hosts, and the frequency of eggs in the environment, in many different regions and situations. These have greatly increased our understanding of the relationship between egg excretion from companion and wild animals and the risk of human infection by inadvertent ingestion of eggs from soil and other environmental reservoirs. Nevertheless, it is difficult to compare studies directly because of vagaries in sampling and laboratory methods, a preponderance of prevalence rather than abundance data, and a lack of studies that systematically sample different sympatric definitive host populations. Such comparisons could be instructive, for example to determine the relative contributions of different definitive host populations and categories to environmental contamination in specified areas, and hence guide priorities for control. In this paper we use estimates of host density and infection levels in the city of Bristol, UK, as a case study to evaluate the relative contribution of sympatric cats, dogs and foxes to overall environmental contamination with eggs. Results suggest that dogs, especially those less than 12 weeks of age, dominate total egg output, but that this is modified by degree of access to public areas and removal of faeces, such that foxes could take over as the primary source of eggs. Results and conclusions are likely to differ among specific locations. The general aim is to show how an improved quantitative framework for epidemiological studies of *Toxocara* spp. egg contamination can help to advance understanding and the effectiveness of control strategies in future.

Keywords: *Toxocara* spp., toxocarosis, egg, soil, distribution, dog, cat, fox, control

Veterinary and public health aspects of *Toxocara* spp.

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Abstract

Pet dogs and cats can play an important role in the transmission of zoonotic nematodes such as *Toxocara canis* and *T. cati*, by excreting eggs directly into the human environment, without the involvement of vectors or intermediate hosts. Human toxocarosis remains a hazard despite the availability of highly effective anthelmintics for dogs and cats. A good understanding of the biology and epidemiology of these parasites, and the risk factors that lead to their transmission to humans is required for effective prevention strategies. In this respect, the maintenance of high quality continuing education for veterinarians and the provision of suitably presented information to pet owners are of priority importance. A closer collaboration between veterinary and public health professionals within the 'One Health' concept is also required.

Keywords: dog, cat, zoonoses, epidemiology, diagnosis, *Toxocara canis, Toxocara cati*, prevention, control

Detection and identification of *Toxocara canis* DNA in bronchoalveolar lavage of infected mice using a novel Real-Time PCR

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Abstract

Toxocarosis is a zoonosis with worldwide distribution caused by *Toxocara* spp. of dogs and cats. In humans, diagnosis relies mainly on detection of parasite-specific antibodies. Although serological assays in current use have defined sensitivity and specificity, the problem of cross-reactivity still remains, particularly in areas of endemic polyparasitism. Microscopic detection of the parasite in tissue biopsies is not recommended for diagnosis because larvae can be difficult to locate, and finding the parasite eggs in faeces is not applicable since the larvae do not develop to the adult stage in the human host. In this study we describe a novel real-time PCR ('Nemo-PCR') that, in combination with DNA sequencing, allows the detection and identification of *Toxocara canis* and other nematodes in the superfamily Ascaridoidea. Results indicate that this approach can detect *Toxocara* spp. DNA in bronchoalveolar lavage (BAL) of experimentally-infected mice. For diagnostic purposes further studies are necessary to evaluate this assay including testing human BAL fluid. The availability of such a direct assay would improve diagnosis of toxocarosis particularly for patients with pulmonary signs and symptoms.

Keywords: *Toxocara canis*, real-time PCR, bronchoalveolar lavage, Ascaridoidea, experimental infection

Toxocara spp. infections in paratenic hosts

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In memoriam Thomas Schnieder

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Abstract

The zoonotic roundworms *Toxocara canis* and *T. cati* are not only present worldwide in their definitive hosts; they also frequently occur in other animal species, including humans. In those so-called paratenic hosts, the larvae do not develop into the adult stage, but rather migrate throughout the somatic tissue and persist as infectious L3 stage for extensive periods. Those arrested larvae may lead to severe inflammatory reactions and consequently to a wide range of pathological and clinical manifestations. However, the infected paratenic hosts also constitute a potential source of infection for the definitive hosts or humans who may also function as paratenic hosts. In the present review, current knowledge of larval migration in a variety of possible paratenic hosts is summarised including variations of migration routes and susceptibilities. Furthermore, information about the clinical and pathological changes for the presented species and possible consequences of the somatic migration of larvae, i.e. the resulting tissue damage as well as adverse host reactions to arrested larvae are reviewed. There are still many questions unanswered regarding larval behaviour in hosts other than their definitive host. Therefore, it is of great importance to continue further elaboration on the biology of *Toxocara* spp. to prevent further spreading of larvae in both the paratenic and the definitive host.

Keywords: *Toxocara cati*, *Toxocara canis*, somatic migration, visceral larva migrans, ocular larva migrans, paratenic host, zoonosis

A retrospective study of the time course of seropositivity to *Toxocara* in a cohort of children treated with anthelmintic in Poland

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Abstract

Toxocarosis is a helminthozoonotic disease caused by ascarid larvae of *Toxocara* spp. The aim of this retrospective study was to monitor the serological response to anthelmintic treatment in a cohort of 103 *Toxocara* seropositive Polish children and the efficacy of treatment when assessed by serology and resolution of clinical signs. Reasons for presentation of the children at hospital included: laboratory abnormalities (eosinophilia, anaemia, antibodies against *T. canis*); children's complaints (abdominal pain, allergic symptoms, headache, loss of appetite, subfebrile conditions, arthralgia); physical examination (enlargement of lymphatic nodes, abdominal tenderness). Seropositive individuals and putative covert toxocarosis were diagnosed in 95.1% of the children and ocular toxocarosis in 4.9%. All the children were treated with antiparasitic therapy (albendazole, albendazole and then mebendazole, mebendazole). After therapy, the mean ELIZA values for *Toxocara* specific antibodies, eosinophilia and the number of complaints from the patients all decreased. The putative *T. canis* infection in children presented with non-specific symptoms. Evaluation of the efficacy of treatment was not easy due to the non-specific symptoms. Lslow kinetics of specific anti-*Toxocara* IgG decrease; nonetheless, continued high specific anti-*Toxocara* IgG values in some children suggested persisting larvae or continued re-infection may have occurred in some individuals.

Keywords: Toxocarosis, *Toxocara* spp., seropositivity, children, Poland

Baylisascariosis – infections of animals and humans with 'unusual' roundworms

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Abstract

The nematode genus *Baylisascaris* (order Ascaridida, superfamily Ascaridoidea) contains nine relatively host-specific, parasite species of carnivores, omnivores, herbivores, carnivorous marsupials or rodents. They have a facultative heteroxenous life cycle, at least under experimental conditions. Eggs passed in faeces embryonate in the environment and the second-stage larva infective for both definitive and intermediate hosts develops. In intermediate hosts larvae migrate extensively through tissues, where they grow and moult to the third-stage, causing extensive damage. All *Baylisascaris* spp. are considered a potential cause of visceral, ocular and/or neural larval migrans in mammals including humans and in birds. This paper summarises our current knowledge on the prevalence, biology, pathogenicity and zoonotic significance of three *Baylisascaris* species: *B. transfuga*, *B. schroederi* and *B. procyonis* which have as definitive hosts bears, giant pandas and raccoons (occasionally dogs), respectively.

Keywords: *Baylisascaris procyonis*, *Baylisascaris schroederi*, *Baylisascaris transfuga*, bear, giant panda, raccoon, larva migrans

Oral Presentation Abstracts

Inhibition of TGF-β1 expression lessens inflammatory injury of experimental pulmonary toxocariasis

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Abstract

Toxocara canis larvae may invade human's lungs to cause pulmonary injury thus resulting in pneumonitis. Since *T. canis* larvae may persist in the lungs to induce various leukocytes infiltrated in the injured sites accompanied with enhanced TGF-β1 expression in experimental pulmonary toxocariasis (PT), whether inhibition of TGF-β1 expression may lessen the inflammatory injury of PT was investigated. The *T. canis*-infected mice were given TGF-β1 inhibitor-LSKL (Leu-Ser-Lys-Leu) peptide twice per day by intraperitoneal injection until 83 days post infection (dpi) to investigate whether inflammatory injury was decreased and the role of pro-inflammatory cytokines such as TGF-β1, TG2, S100B, PCNA, eotaxin, and SP in mice with PT at 2 and 83 dpi. Pathologically, *T. canis* larval invasion of the lungs might cause pulmonary injury with mild leukocytes infiltration at 2 dpi and then the inflammatory injury became more severe at 83 dpi. Pro-inflammatory cytokines expressions were also increased from 2 to 83 dpi. However, less pulmonary injury accompanied with decreased pro-inflammatory cytokines expressions in LSKL-treated mice was found as compared to normal saline-treated group at 2 and 83 dpi. In conclusion, reduction of TGF-β1 expression can lessen inflammatory injury of experimental pulmonary toxocariasis.

Clinical and laboratory correlates of human toxocarosis diagnosed by indirect immunofluorescence

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Abstract

Purpose: Surveillance of serologically positive patients on toxocarosis and dynamics of antibody levels were performed up to ten years after diagnosis.

Methods: An antigen was produced for an indirect immunofluorescence test for serological diagnosis in humans using *Toxocara canis* larvae harvested from the brains of laboratory rodents. This antigen enables the detection not only somatic but excretory-secretory *Toxocara*-antibodies in human sera.

Main findings: We found a few hundred positive patients and distinct cases of visceral and ocular disease (1). Respiratory involvement, hypersensitive dermatitis, and neurological symptoms with high blood eosinophilia, especially in children, were recorded. In healthy blood donors, we found 6% of samples positive for *Toxocara* antibodies. In differential diagnostics of visceral larva migrans syndrome, we identified a case of pulmonary capillariosis (2). Albendazole therapeutic protocol of 15mg per kilogram of body weight for 30 days is used to prevent ocular or brain invasion by *Toxocara* larvae.

Conclusion: One to two years after therapy, eosinophilia and antibody levels significantly decreased, in comparison with non-treated group, where both eosinophilia and antibodies marked activity of infection.

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Short history and review of relevant data of serodiagnosis of human toxocarosis in Hungary

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Abstract

The serodiagnosis of human toxocarosis of suspected patients was introduced in Hungary in the mid 70's at the Department of Parasitology's National Centre for Epidemiology. The first time the larval microprecipitation test was applied, which had been replaced after 10 years by a home-made ELISA utilising larval excretorysecretory antigens. In parallel with the spread of commercial products the serodiagnosis has been decentralised. At our Department, the home-made ELISA was replaced by a commercial one in 2002, followed by application of a commercial Western blot for verification purposes 1 year later.

Reviewing just the computer assisted era of patients' records (since 1993 to 2011) nearly 40,000 people have been examined for specific antibodies with different tests, with a yearly positivity rate between 16 and 35%. In a nationwide serological survey, 6,985 healthy asymptomatic individuals, representing different age groups and different regions of Hungary were screened for antibodies with another ELISA in 2000. Surprisingly the overall seropositivity rate 28% was found almost as high as in the diagnostic population with clinical signs. On the occasion of this contradiction some aspects of manufacturer specific settings of cut-off levels and interpretations are discussed, and the need for some standard preparations to solve these comparability problems are also emphasised.

Migration and survival of *T. canis* larvae in eosinophil-deficient mice

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Infection with parasitic helminths often induces peripheral eosinophilia, and this immune response was historically thought to confer protection to the host. However, recent work on *Trichinella spiralis* infection in mice showed that larval growth and survival were impaired in the absence of host eosinophils. In a preliminary study to determine if this held true for other helminth larvae, eosinophil-deficient ΔdblGATA-/- mice and C57BL/6 control mice were inoculated orally with 125 embryonated *Toxocara canis* eggs, followed by another 500 eggs 28 days post-infection (dpi). At 1, 3, 5, 7, 29, 31, 33, 35, 37, and 39 dpi, one mouse from each group was euthanised and the following body compartments were separately digested in pepsin-HCl solution: liver, heart+lungs, brain, upper body, and lower body. Samples were then examined microscopically for *T. canis* larvae. Larval migration pattern and timing were essentially the same for both groups. Interestingly, the total number of recovered larvae was lower in eosinophil-deficient mice than in control mice, particularly at later timepoints (37 and 39 dpi). Preliminary findings suggest that, as with *T. spiralis*, eosinophils may play a supportive role in *T. canis* larval survival. This effect is likely to become more pronounced with time after infection. Further experiments are planned to help verify these results in more chronically infected mice.

Simulation of antibody responses to *Toxocara:* a modelling approach

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Abstract

Sero-prevalence data clearly illustrate that human exposure to *Toxocara* is frequent. Environmental contamination with *Toxocara* spp. eggs is assumed to be the best indicator of human exposure, but increased risk of exposure has also been associated with poverty, poor hygiene, age, gender, infection rates in dogs in the domestic environment and many other factors. Moreover, reported associations may be confounded by unobserved risk factors, often hampering an objective interpretation of factors driving the onset of antibody positivity to *Toxocara*. The objective of the work presented is to assess the validity of our current conceptual understanding on the key processes driving exposure to human toxocariasis. We constructed a rule-based model predicting antibody positivity to *Toxocara* in children, using environmental contamination with *Toxocara* spp. eggs and age-related risk of infection as main determinants. The results of the simulation were validated with published data from 5 different environmental contexts: Brazil (2 different locations), Argentina, Poland and the Netherlands. Using simple rules and a stochastic approach with parameter estimates derived from the respective contexts, we succeeded in predicting antibody responses to *Toxocara*. Our approach leads to novel insights in the dynamics of antibody responses to *Toxocara* and the interplay between immune responses and force of infection. Results will be discussed and future applications proposed.

Poster Presentation Abstracts

Winner of the prize for the poster presentation which best demonstrated excellent scientific merit at the ESCCAP *Toxocara2012* meeting in Budapest, Hungary. This award was made in memory of Professor Thomas Schnieder.

Enhanced expressions of brain injury-associated biomarkers and impairment of UPS during acute infection of *Toxocara canis* in mice.

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Abstract

During brain injury (BI), expression of BI-associated biomarkers (BIABs), including TGF-β1, S100B, GFAP, NF-L, tTGs, AβPP, and tau are enhanced and the UPS is impaired. Larvae of *Toxocara canis* can invade the brain and cause BI in humans and mice, leading to cerebral toxocariasis (CT). To understand the pathogenesis of CT in human, we adapted murine toxocariasis as an experimental model. BIAB expressions and UPS function in the brains of mice inoculated with a single dose of 250 *T. canis* embryonated eggs was investigated from 3 days (dpi) to 8 weeks post-infection (wpi). Results revealed that at 4 and 8 wpi, *T. canis* larvae were found to invade areas around the choroid plexus but without eliciting leukocyte infiltration in brains of infected mice; nevertheless, astrogliosis, an indicator of BI, with 78.9~142.0-fold increases in GFAP expression was present. Meanwhile, Western blotting showed markedly increased levels of TGF-β1, S100B, NF-L, tTG, AβPP, and tau(2.0~12.0-fold), although their mRNA expressions were not consistent at 8 wpi. Concomitantly, UPS impairment was evidenced by the overexpression of conjugated ubiquitin and ubiquitin in the brain. Increased levels of neurodegeneration-associated AβPP and phosphorylated tau emerged in the brain may lead to an increased risk of CT progression into neurodegenerative disease.

Winner of the prize for the poster presentation which best demonstrated excellent communication across scientific disciplines at the ESCCAP *Toxocara2012* meeting in Budapest, Hungary. This award was made in memory of Professor Huw Smith.

Migratory pattern of *Toxocara cati* and *Toxocara canis* in pigs

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Abstract

In man infections with *Toxocara cati* and *T. canis* may cause visceral larva migrans (VLM). The relative contribution of the two species in VLM is largely unknown, as well as the predilection sites of *T. cati*. We used pigs as a model for human infection to explore the migratory patterns of *T. cati* as compared to *T. canis*. Two groups of 7 pigs were inoculated with 10.000 *T. cati* and *T. canis* eggs, and 4 pigs remained uninfected. Larvae were recovered from the liver, lungs, mesenterial lymph nodes, heart, brain, eyes, diaphragm, tongue and skeletal muscles at day 30-32 post infection by digestion. Larval recovery was low in both groups (<0.4% of infection dose). More larvae were found in lymph nodes of *T. cati* than *T. canis* (3.4 vs. 1.3; p=0.07), but not significantly so. The opposite trend was found in the lungs (1.9 vs. 14.6; p=0.07). Two larvae were recovered from skeletal muscles of a *T. cati*-infected pig and one larva from the diaphragm and liver of two *T. canis*-infected pigs. Haematology showed more pronounced eosinophilia in *T. canis* compared to *T. cati*-infected pigs. Our data suggests a different tissue distribution of *T. cati* and *T. canis* which may reflect different migratory patterns in VLM and perhaps different pathogenicity.

This work was supported by grants from the Japan Society for the Promotion of Science (Young Researcher Overseas Visits Program for Vitalizing Brain Circulation, S 2213).

Seroprevalence of *Toxocara canis* infection among schoolchildren in the Democratic Republic of São Tome è Principe (DRSTP), West Africa

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Abstract

This study intends to investigate the status of *Toxocara canis* infection among schoolchildren in the Democratic Republic of Sao Tomes è Principe (DRSTP), West Africa. In total, 540 blood samples were randomly collected from children (9.0 ± 1.6 yr old) from 10 primary schools located in three provinces of Aqua Grande, Me Xozi and Lobata, respectively after obtaining informed consent from parents or guardians from 2010 to 2011. *T. canis* infection was examined by detection of sera immunoglobulin G (titer set at ≥1: 64) using a *T. canis* larval excretory-secretory antigen-based Western blot assay. The overall seroprevalence was surprisingly high, reaching 96.7% (522/540). No statistical difference was found between boys (96.6%, 259/268) and girls (96.7%, 263/272) (p = 0.97). Geographically, Aqua Grande had significantly higher prevalence (252/255, 98.8%) than that in Me Xozi (137/145, 94.5%) (p = 0.01) or Lobata (133/140, 95.0%) (p = 0.02). The high seroprevalence of *T. canis* infection among schoolchildren may be due to constant exposure to the parasite from their regular activities with poor personal hygiene and environmental sanitation; in addition, whether there was a high frequency of seizures present in schoolchildren should be further evaluated in DRSTP.

Influence of *Toxocara canis* glycans on *in vitro* proliferation and cytokine production by mouse spleen cells

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Abstract

Nematode glycans play an important role in regulation of host immune response. Glycans present in *Toxocara canis* excretory-secretory glycoproteins have been characterised mainly as O-methylated trisaccharides (Khoo et al. 1991, Glycobiology 1, 163-171). It has been proven that they are specific targets for antibody binding (Schabussova et al. 2007, Int J Parasitol 37, 97-109) but there is no information about their role in regulation of T cell activity.

The aim of the study was to investigate the influence of *T. canis* ES glycans on *in vitro* proliferation and production of cytokines by mouse splenocytes. Cells were stimulated with native ES products containing intact glycans or ES products deglycosylated by metaperiodate oxidation. Levels of Th1, Th2 and Th17 cytokines were measured in culture media by commercial ELISA. We have noted changes in proliferation and cytokine profile which suggest that glycan parts of glycoproteins are important in regulation of the cellular response.

This work was supported by the Polish National Science Centre (grant no. N N308 573540)

The prevalence of *Toxocara* spp. in sandpits of children's playgrounds in the city of Hanover

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Abstract

Toxocara spp. are worldwide distributed parasites which can cause zoonotic diseases in humans. The present study was conducted to provide evidence for the occurrence of parasite stages on public places in Hanover and estimate the infection risk for humans, especially children, by contaminated soil or sand, respectively. In the year 2011, sand samples were collected from 46 sandpits in different playgrounds in the city of Hanover. Sand samples were taken monthly from January to December, dried at 37 °C followed by determination of the degree of moisture. Of each sand sample, 250g were processed by an optimised flotation-sedimentation technique followed by microscopical examination for parasitic objects. These were counted and differentiated for the genus or species, respectively. Preliminary results show, investigation of sandpit samples from January, April, July, October and December resulted in a percentage of 8.70% (December) to 28.26% (April) ascarid egg-positive playgrounds. Thereby, eggs of *Toxocara* spp. were found in 6.52-28.26% of the samples, whereby embryonated eggs were present in 0-28.26%. The number of eggs varied between 1-28 per 250g sand sample. Further work will include examination and statistical analysis of the remaining months and results will be discussed.

Larval distribution and neuropathological changes in brains of paratenic hosts after *Toxocara* spp. infection

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Abstract

The worldwide distributed roundworm *Toxocara canis* causes zoonotic infections due to persistence of infectious larvae in the paratenic host's tissue. Former studies have shown that *T. canis* has an increased neural affinity whereas little is known about the distribution of *T. cati* in the paratenic host. Therefore, larval distribution in the mouse as a model for paratenic hosts was evaluated for both, *T.* canis and *T. cati* by artificially digesting organs and determining the number of larvae microscopically at eight time points post infection, mainly focusing on the brain. Additionally, two brains per group were examined histopathologically to observe possible changes in the brain and possible differences between the study groups. Preliminary results indicate differences in migration behaviour between the two *Toxocara* spp. Determination of maximum larval counts in brains as well as distribution of larvae in organs are currently in progress. Analysis of the brain at different time points will provide important data on whether *T. cati* and *T. canis* arrest in the brain or if they continue migration and how the brain is affected. Obtained data will generate a profile of *T. cati* compared to *T. canis* larvae in the paratenic host and provide an inevitable foundation for further research on toxocarosis.

Activity of a herbal blend on the control of *Toxocara canis* in dogs: preliminary results.

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Abstract

The use of medicinal plants combined with routine antihelminthic treatments could enhance the control of *Toxocara canis*, which represents the most prevalent intestinal helminth in dogs. The objective of this study was to evaluate the efficacy *in vivo* of an oral supplementation of a herb blend in a canine population at risk. Sixteen adult dogs owned by 2 hunters were used. Dogs were first dewormed (12,5mg milbemycin, 125mg praziquantel) and allocated into 2 groups: Control, C (n=7) or Supplemented, S (n=9). Throughout the whole study, the C group received a complete dog food while the S group received the same diet with 0.17% herbal blend (*A. sativum*, *M. piperita*, *U. fulva, T. vulgaris*, *G. aperine*, *U. dioica*, *P. excelsa*, *C. minimum*, *C. zelandicum, F. vulgare*). Faecal samples were collected at 6 and 8 months following the deworming treatment and quantitative and qualitative coprological assays were carried out. After 8 months, 25% of the dogs in C group were positive to *T. canis*, while all dogs remained negative in S group (P=0.009). Faecal Egg Count was significantly higher in C group than in S group (60 epg vs 0 epg, P=0.012). This herbal mix could help to control *T. canis* infection between deworming intervals that are too long.

Supported by Affinity Petcare.

Significance of Border-line Antibody Level in Human Toxocarosis – Comparison of Three Serologic Tests

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Abstract

Purpose: In clinical diagnostics of human toxocarosis, a great problem are low ELISA values near to limit of significance. Decision about anthelminthic therapy of patients with borderline antibody level is very important medical and ethical question.

Methods: The importance of boundary values of antibody level on toxocarosis have been examined by using two serological tests, ELISA (NovaTec Immunodiagnostica GmbH and TEST-LINE Clinical Diagnostic, Brno, Czech Republic) and immunofluorescent assay (performed with "home-made" antigen production, as histological slides of brains of artificial infected mice) and results have been confirmed by western-blot method (DIAMEDIS Laboratory Diagnostische Medizin in Sennestadt). All negative sera but close to borderline must be tested in ELISA of other producer and/or immunofluorescent assay and western-blotting.

Main findings: Results of ELISA and immunofluorescent assay were concordant in more than 90% of patients' sera. But, of 70 samples, we identified two sera negative or borderline in ELISA of one producer and positive of another producer, and six sera negative in ELISA and positive in immunofluorescent assay. In one of these patients, in liver biopsy, granuloma with dead larva resembled *Toxocara* was found. Low level of antibodies suggest infection by low number of *Toxocara* larvae, when risk of ocular or neural localisation increasing then in classical larva migrans syndrome.

Conclusion: Patients must be tested by different serological methods and if they are positive in one, anthelminthic therapy must be recommended.

Toxocara spp. detection in public parks from Madrid, Spain

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Abstract

Several studies have demonstrated that the soil of public parks presents an important source of infection which has a significant impact on public health as *Toxocara* spp. may cause larva *migrans* visceral/ocular in humans. The aim of the present study was to identify the presence of *Toxocara* spp in soil and faecal samples contaminating public parks of Madrid. Two different socio-economical areas were evaluated: Pozuelo de Alarcón (PA) (socio-economic status high-medium) and Vallecas (VV) (socio-economic status medium-low). Several soil and faecal samples were collected and performed with routine coprological methods (Telemann sedimentation modified method). A total of 67 and 43 public parks from VV and PA, respectively, were included in the present study. *Toxocara* spp. eggs were detected in soil samples from VV (16.4%) and PA (11.6%), while *Toxocara* spp. was only detected in 4.9% faecal samples (4/81) from PA. Besides, other intestinal parasites were detected in soil samples: Fam. Ancylostomatidae (3% in VV and 9.3% in PA), *Toxascaris leonina* (2.3% in PA), *Cystoisospora* spp. (4.6% in PA) and *Giardia duodenalis* (4.5% in VV). In faecal samples were also detected intestinal parasites: *D.caninum, T.leonina, T.vulpis,* Fam. Ancylostomatidae*, Cystoisospora* spp*.* and *G.duodenalis*. It is remarkable the high prevalence of zoonotic parasites detected in soil and faecal samples, being one of the most prevalent *Toxocara* spp. Statistical differences were not observed between those two different socio-economic areas.

Temporal trends and age-related patterns of human toxocariasis: a retrospective study in Belgium (2000-2011)

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Abstract

Sero-prevalence data place human toxocariasis, mostly attributed to infection with *Toxocara canis*, among the most common zoonotic helminth infections worldwide. Nevertheless, the disease remains neglected and information on its distribution and dynamics is still lacking in many countries. This is also the case in Belgium.

We conducted a retrospective study on available data of a cohort of subjects suspected of human toxocariasis who were tested for *Toxocara* antibody seropositivity in the period from January 2000 to March 2011 at the Institute of Tropical Medicine in Antwerp. The complete cohort included 7712 patients, between 0 months and 94 years of age. An overall antibody seropositivity rate of 7.2% was measured, with no significant changes across the study period. Age-related patterns in seropositivity showed a binomial distribution with a higher proportion of antibody-positive subjects below the age of 5 and above the age of 40 years. This study provides novel information on temporal trends and distributions of human toxocariasis in Belgium. In addition, further analysis of this large dataset clearly demonstrated the importance of age-related patterns in antibody seropositivity to *Toxocara*. The identification of the mechanisms underlying the distributions and dynamics of *Toxocara* antibody responses will provide much needed insight into the epidemiology and transmission of human toxocariasis.

Serological diagnosis of Ascarid Visceral Larva Migrans with recombinant antigens

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Abstract

The diagnosis of ascarid Visceral Larva Migrans Syndrome (VLM) relies almost exclusively on serological tests. However, it is often unable to discriminate between toxocariasis and ascariosis, due to cross-reactivity between *Toxocara sp.* and *Ascaris sp.* antigens. In this study, we evaluated two antigens, recombinant *T. canis* antigen (rTc) and recombinant As16 (rAs16) of *A. suum*, for the diagnosis of ascarid VLM in humans. ELISA was performed with 120 serum samples from suspected ascarid VLM patients, who were seropositive for *T. canis* and/or *A. suum* L3 ES antigens (Tc-ES, As-ES). Of these 120 samples, 78 samples (65.0%) showed positive binding to rTc and/or rAs16. Then, patients were considered as having toxocariasis or ascariosis, if the ratio of optical densities between Tc-ES and As-ES or between rTc and rAs16 were more than 2.0. Although ES antigens produced ambiguous results in 47 of 120 cases (39.2%), 51.1% of these ambiguous 47 cases were discriminated with rTc and rAs16 as toxocariasis or ascariosis. Present study indicates that rTc and rAs16 are more specific than ES antigens in ELISA. The combination of ES antigens and these recombinant antigens ELISA would provide us with more reliable results in the diagnosis of ascarid VLM.

This work was supported by grants from the Japan Society for the Promotion of Science (Young Researcher Overseas Visits Program for Vitalizing Brain Circulation, S 2213) and the Ministry of Health, Labour and Welfare (H22 Seisaku-Souyaku-Ippan-003).

Seroprevalence of human toxocariasis in Slovenia

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Abstract

Human toxocariasis is a zoonosis caused by larvae of the dog roundworm *Toxocara canis* or the cat roundworm *Toxocara cati*. Diagnosis of the infection relies mainly on serology. In the present study, the seroprevalence of *Toxocara* was assessed from 2963 suspected patients whose serum samples were sent to our institute from the beginning of January 2003 to the end of March 2012. Of 505 enzyme-linked immunosorbent assay-positive or equivocal serum samples, 280 were confirmed positive by Western blot. Hence, the overall seroprevalence among individuals included in the study was 9,4%. The mean seroprevalence in the first five study years (2003-2007) (11,9%) was significantly higher ($p<0,05$) than in 2008-2012 (6,1%). There was no statistically significant difference in the seroprevalence between genders but the seroprevalence shows a significant increase with age, ranging from 3,5% for the age group 0-9 to 19,4% for the age group 60-69. Since there is no other laboratory performing serology testing for *Toxocara* in Slovenia, the study is an overview of the situation concerning the seroprevalence for these roundworms over the past 9 years and 3 months in our country.

Treatment of third stage larvae of *Toxocara cati* with milbemycinoxime/praziquantel tablets and emodepside/praziquantel spot-on in experimentally infected cats

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Abstract

Cats of all age groups are frequently infected with *Toxocara cati*. For successful treatment it is beneficial to not only eliminate intestinal stages but also development stages within the tissue. A study was conducted to assess the efficacy of milbemycinoxime/praziquantel tablets (Milbemax®, Novartis) against third stage larvae of *T. cati* in comparison to the positive spot-on control product emodepside/praziquantel (Profender®, Bayer). 24 kittens were experimentally infected with *T. cati* and randomly allocated to three study groups. Five days after the experimental infection the cats were treated with the minimum therapeutic dosage. The development of patent infections was monitored and all cats were dewormed 50 days after the experimental infection. To calculate the efficacies, the counts of excreted worms in the treated groups were compared to the negative control group. In the negative control group 7 of 8 cats developed a patent *T. cati* infection and all 8 cats excreted worms after deworming (geometric mean worm count 18.1). For the milbemycinoxime/praziquantel treated animals no efficacy could be observed. All 8 cats developed a patent infection and excreted worms (geometric mean worm count 27.7). The efficacy of the emodepside/praziquantel spot-on formulation was 98.5% against L3 of *T. cati*. One cat developed a patent infection and excreted worms after deworming (geometric mean worm count 0.3).

Epidemiologic and clinical aspects of toxocariasis in children

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Abstract

Toxocara spp. infection in humans can be asymptomatic or result in two main clinical syndromes: visceral larva migrans and ocular larva migrans. The aim of the study was analysis of clinical manifestations in children with confirmed *Toxocara* infection. From 1.01.2010 to 30.06.2012 toxocariasis was diagnosed in 77 children. The diagnosis was confirmed with a positive result of the enzyme-linked immunosorbent assay with excretory-secretory *Toxocara spp*. antigens. We analysed history, signs and symptoms, abnormalities in physical and laboratory examinations. The children with toxocariasis,45 boys and 32 girls, were 1-17 years old (median age 9 years). 53 children lived in urban and 24 in rural areas, 32 had direct regular contact with dogs or cats, one child had history of pica. Children with toxocariasis suffered from: abdominal pains (15), recurrent respiratory infections or persistent cough (11), failure to thrive (6), unilateral vision impairment (5), exacerbation of allergic disorders (4), headache (3), fever (2). Physical examination revealed lymphadenopathy in 11 children, hepatomegaly in 6, splenomegaly in 4. Unilateral ocular lesions (granuloma, chorioretinitis, optic neuritis) were found in 5 children. Lab tests showed eosinophilia in 43 children, increased IgE levels in 14 and profound anaemia in 2. Typical visceral or ocular larva migrans are rare, more commonly clinical manifestations of toxocariasis are subtle and non-specific.

Severe anaemia in the course of toxocariasis - case report

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Abstract

Infection with *Toxocara spp.* in children is usually asymptomatic. In some cases it causes a variety of manifestations and the clinical course can be severe. We present a case of profound anaemia caused by *Toxocara* infection. A 3½ year old girl, previously healthy, living in rural area, exposed to dogs and cats, was admitted to the hospital because of anaemia. Physical examination revealed pallor, tachycardia, heart murmur and hepatosplenomegaly. Lab tests showed profound anaemia (Hgb 5,1g/dl), leukocytosis (20,1cells/mcL), eosinophilia (46%), iron deficiency and hypergammaglobulinemia. Hypoechogenic lesions were found in the liver by ultrasound. Bone marrow examination revealed eosinophilia (27,2%) and suppression of erythropoesis. Leukaemia was excluded. Result of the enzyme-linked immunosorbent assay (ELISA) with excretory-secretory *Toxocara* antigens was positive. Fundoscopic examination showed no abnormalities. Visceral larva migrans syndrome was diagnosed and a 3-week course of diethylcarbamazine was used. Three weeks later hepatosplenomegaly and liver lesions resolved, haemoglobin level increased (9,6g/dl), eosinophilia (52%) persisted. After 5 months allergic exanthema occurred. Lab tests revealed increase in haemoglobin level (10,2g/dl), decrease in eosinophilia (26%) and ELISA titer. Urticaria was diagnosed, not related with toxocariasis. Two years after treatment haemoglobin level was normal, moderate eosinophilia (17%) was still present, with no abnormalities on physical and fundoscopic examination. Visceral larva migrans syndrome should be differentiated with proliferative disorders. Eosinophilia can persist for months.

A phylogenetic study on nad1 gene sequence of ascaridoid nematodes isolated from dogs and cats in Iran

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Abstract

The present study determined the phylogenetic position and sequence variability among and within different geographical isolates of *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina* isolated from dogs and cats in Iran using sequence data for mitochondrial DNA gene, the subunit 1 of NADH dehydrogenase gene (nad1). The nad1 gene was amplified by polymerase chain reaction using the primer set ND1FND1R. Seven *T. canis,* 26 *T. cati* and 4 *T. leonina* were sequenced and aligned using Bioedit software and compared with published sequences in GenBank. Phylogenetic analysis was performed using MEGA 4.0 software. The sequences of nad1 gene were 366 bp in length. A pair wise nucleotide sequence analysis of the nad1 gene of the tested isolates demonstrated high intra-species diversity for *T. canis* (0-1.7%) and *T. cati* (0-2%) in comparison with *T. leonina* (0-0.6%). The inter-specific difference between *T. canis* and *T. cati* was significant (13.4-16.4%), while the inter-specific sequence differences between *Toxascaris* and *Toxocara* was significantly higher,

being 25.5-34.7%. Sequencing and phylogenetic analyses of the nad1 gene indicated that there was genetic diversity among the isolates of *T. canis*, *T. cati* and *T. leonina* from different areas of Iran.

Wild carnivores as key hosts for the maintenance of *Toxocara* spp. in Portugal

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Abstract

Toxocara canis and *T. cati* are the only *Toxocara* species described in Portugal. Authors review the relevance of wild carnivores for the epidemiology of toxocarosis in Portugal. Red foxes are the most important wild hosts for *T. canis* and a synanthropic cycle between dogs, rodents and foxes may exist in periurban areas. Despite decreasing prevalence in dogs, prevalence in foxes has been constant, with an increasing tendency (11% in the 1970s; 16-37% in the 2000s). Recent data show that wolf populations have *Toxocara canis* prevalence around 7%. Stray cats exhibit the highest infection rates by *T. cati* (10.4%) and may be more important than wild felids in maintaining the infectious pressure. Little is known about the role of small wild carnivore species in the epidemiology of toxocarosis. Data suggests a minor importance, but in other countries high prevalence was found for the Egyptian mongoose. This invasive species in the Iberian Peninsula is ecologically similar to the red fox and further studies will allow us to fully understand its role. Concluding, *Toxocara* spp. shows a growing prevalence in wild carnivores and its interaction with domestic carnivores should be better understood at the epidemiology and control levels.

Environmental disinvasion as a basis of preventive measures against toxocariasis

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Abstract

The paper objective was to carry out a helminthological study and to create an up-to-date technology that would allow to perform disinvasion in order to decontaminate environment from parasitosis agents. Sanitary-parasitological study of soil, sand and bottom silt were performed in accordance with Methodological Guidelines (MG) 4.2.2661-10 "Methods of sanitary parasitological study" and MG 3.2.1756 –03 "Epidemiological surveillance of parasitic infections". The soil study showed that *Toxocara* eggs were found in 9.7% of sand and soil samples. There were predominantly viable eggs in all contaminated specimens. The study of anonymous faecal specimens showed that *Toxocara* eggs were found in 39.5% of samples. The egg contamination intensive indices varied between 300 and 1000 detections per kilogram. Preventive measures against parasitic diseases is to efficiently perform environmental disinvasion. Purolat-Trade Ltd. has developed an ecologically safe vegetational ovicidal product Purolat-Bingsti. Purolat-Bingsti by biological inhibiting and stimulation causes the natural death of helminth eggs. It has no effect on metabolism of active sludge biocoenosis, soil or human health. Helminth ova are deprived of their invasive capacity and no longer represent any epidemiological risk and are not able to cause helminths infection among humans or animals. Purolat-Bingsti's efficiency of wastewaters and sludge disinvasion is 95.5-99%.


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