

№5

Binev R, Kirkova Z, Nikolov J, Russenov A, Stojanchev K, Lazarov L, Hristov T. Efficacy of parenteral administration of ivermectin in the control of strongylidosis in donkeys. *J S Afr Vet Assoc.* 2005 Dec;76(4):214-6.

Investigations into the efficacy of parenteral ivermectin (Pandex) administration for strongylidosis control in donkeys were carried out. The preparation was applied subcutaneously at a dose of 0.2 mg/kg (1 ml/50 kg body weight). One day prior to the treatment and 14 days post-treatment, individual coprological samples were obtained for faecal nematode egg counts and larval culture. The study was performed on 263 donkeys originating from different regions of Bulgaria. Prior to the treatment and 20 days after that, blood samples were obtained from 64 previously infected animals for monitoring of changes in eosinophil leukocyte counts. The subcutaneous application of ivermectin had an efficacy of 96% in terms of reduction of faecal egg counts. In 92.2% of infected donkeys, a complete reduction of faecal eggs count occurred (0 eggs per gram of faeces epg), whereas in the remaining 7.8% of the infected donkeys, the egg counts were reduced by 72%. The reduction in faecal egg counts did not result in changes in eosinophil counts. The results obtained as well as the lack of local changes after the subcutaneous application of ivermectin in donkeys allow us to recommend its use for control of strongyles in donkeys.

№6

Casulli A, Interisano M, Sreter T, Chitimia L, Kirkova Z, La Rosa G, Pozio E. Genetic variability of *Echinococcus granulosus sensu stricto* in Europe inferred by mitochondrial DNA sequences. *Infect Genet Evol.* 2012 Mar;12(2):377-83.

The genetic diversity of *Echinococcus granulosus sensu stricto* (s.s.) metacestodes from four European countries was evaluated by the DNA sequence analysis of the cytochrome c oxidase subunit 1 (cox1) mitochondrial gene. Of the 312 organisms investigated, 132 were from Bulgaria, 35 from Hungary, 89 from Italy and 56 from Romania. Considerable intraspecific variation was observed in the mitochondrial cox1 sequences: 24 haplotypes were detected in the Eastern European population and seven in the Italian population. The Eastern European population parsimony network displayed a star-like features consisting of the most common haplotype EG1 (G1 genotype) and the three major haplotypes: EG2, EG3 and EG4. The EG1 was also the major haplotype in the Italian population network, though with a higher prevalence (73%) compared to the Eastern European network. The percentage of the population constituted by the G1 genotype was used as an indirect index to evaluate the genetic diversity within *E. granulosus* s.s. populations of Eurasia. A clinal correlation between the percentage of the G1 genotype and the geographical regions of Eurasia was observed: the G1 genotype is highly represented in the Mediterranean Basin; it decreases in Eastern Europe and South-West Asia and increases in China. This clinal correlation could reflect the spreading of livestock domestication from Southern-Western Asia during the Neolithic period, beginning around 12,000 BC.

№7

Ivanov. A, Z. Kirkova, P. Iliev, K. Uzunova. A Case of *Pentastomum denticulatum* Infection in Goats. *İstanbul Üniv. Vet. Fak. Derg. J. Fac. Vet. Med. Istanbul Univ.* 38 (2), 191-195, 2012

A case of *Pentastomum denticulatum* infection in goats was described. In August 2007, in a herd of 30 goats, in the region of Rousse (North Bulgaria), there were observed non-specific clinical signs: reduced appetite, depression, emaciation, lying down, and decreased milk secretion. Despite antibiotic therapy 3 of the goats died. A necropsy was performed and small, yellow-white oval cysts with a white parasite inside were established in liver, lungs and mesenteric lymph nodes. The parasites (total number - 30) were examined microscopically and determined as *Pentastomum denticulatum* – larval stage of *Linguatula serrata*.

№8

Rehbein S, Capári B, Duscher G, Keidane D, Kirkova Z, Petkevičius S, Rapti D, Wagner A, Wagner T, Chester ST, Rosentel J, Tielemans E, Visser M, Winter R, Kley K, Knaus M. Efficacy against nematode and cestode infections and safety of a novel topical fipronil, (S)-methoprene, eprinomectin and praziquantel combination product in domestic cats under field conditions in Europe. *Vet Parasitol.* 2014 Apr 28;202(1-2):10-7.

A novel topical combination product (BROADLINE®), Merial) composed of fipronil, (S)-methoprene, eprinomectin and praziquantel was evaluated for safety and efficacy against nematode and cestode infections in domestic cats. The study comprised a multi-centre, positive control, blinded, field study, using a randomized block design based on order of presentation for allocation. In total 196 client-owned cats, confirmed as positive for naturally acquired infections of nematodes and/or cestodes by pre-treatment faecal examination, were studied in seven countries in Europe. Pre-treatment faecal examination revealed the presence of *Toxocara*, hookworm, *Capillaria* and/or spirurid nematode infections in 129, 73, 33 or 1 cat(s), respectively; infections with taeniid and *Dipylidium* cestodes were demonstrated in 39 and 17 cats, respectively. Cats were allocated randomly to one of two treatments in a ratio of 2, topical fipronil (8.3%, w/v), (S)-methoprene (10%, w/v), eprinomectin (0.4%, w/v) and praziquantel (8.3%, w/v) (BROADLINE®), Merial; 130 cats); and 1, topical PROFENDER® Spot-On (Bayer; 66 cats) and treated once on Day 0. For evaluation of efficacy, two faecal samples were collected, one prior to treatment (Day -4 ± 4 days) and one at the end of the study (Day 14 ± 5 days). These were examined for fecal forms of nematode and cestode parasites. For evaluation of safety, cats were examined by a veterinarian before treatment and at the end of the study, and cat owners recorded the health status of their cats daily until the end of the study. For cats treated with Broadline®, the efficacy was >99.9%, 100%, and 99.6% for *Toxocara*, hookworms, and *Capillaria*, respectively; and the efficacy was >99.9%, >99.9%, and 98.5%, respectively, for the cats treated with Profender® ($p < 0.001$ for all nematodes and both treatments). Efficacy was 100% for both cestodes for both treatments ($p < 0.001$). No treatment related adverse experiences were observed throughout the study. For both treatments, every cat that completed the study was given a safety score of 'excellent' for both local and systemic evaluations. The topical combination product of fipronil, (S)-methoprene, eprinomectin and praziquantel was shown to have an excellent safety profile and demonstrated high levels of efficacy when administered once as topical solution to cats infected with nematodes and cestodes under field conditions.

№9

A. Rusenov, L. Lazarov, Z. Kirkova, A. Tonev, N. Rusenova, F. Dilda, 2015. Transient glomerular dysfunction in dogs caused by *Dirofilaria immitis* infection. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 21, 1, 117-121.

The present study aimed to examine two glomerular markers (urinary albumin, uALB; urinary C-reactive protein, uCRP) in healthy dogs and in dogs infected with *Dirofilaria immitis*, and to identify some possible changes in these markers after therapy with ivermectin and doxycycline. Twenty dogs with *D. immitis* infection positive by both the Knott method and SNAP 4Dx (IDEXX, USA) test were included in the research, as well as twelve clinically healthy dogs of similar age which served as controls. Glomerular biomarkers (mean, SD) increased significantly in dogs with heartworm disease (uAIB/Creatinine, Cr mg/g: 527.57 ± 312.54 ; uCRP/Cr mg/g: 0.520 ± 0.624), compared to control dogs (uAIB/Cr mg/g: 94.44 ± 56.50 ; uCRP/Cr mg/g: below detection limit). Six months after the initial examination and the simultaneous treatment, all glomerular markers were considerably decreased and did not differ from those in healthy animals.

In conclusion, the observed changes in glomerular biomarkers clearly indicated the transient nature of glomerular dysfunction caused by the heartworm infection in dogs, which may be of clinical relevance.

№10

M. F. Sommer , R. Beck , M. Ionita, J. Stefanovska, A. Vasić, N. Zdravković, D. Hamel, S. Rehbein , M. Knaus , I. L. Mitrea , E. Shukullari, Z. Kirkova, D. Rapti, B. Capári, C. Silaghi. Multilocus sequence typing of canine *Giardia duodenalis* from South Eastern European countries. *Parasitology Research* 114(6) · March 2015

Giardia duodenalis is a worldwide occurring protozoan that can infect various mammalian hosts. While living conditions are getting closer between pet animals and owners, there is discussion whether dogs may contribute to the transmission of these pathogens to humans. The present study was conducted in order to identify the *Giardia* assemblages in dogs from South Eastern Europe. For this purpose, 1645 faecal samples of household and shelter dogs from Albania, Bulgaria, Hungary, Macedonia, Romania and Serbia were tested for *Giardia* coproantigen by enzyme-linked immunosorbent assay (ELISA). A subset of 107 faecal samples demonstrating *Giardia* cysts by direct immunofluorescence assay (IFA) or microscopy (15-22 per country) plus 26 IFA-positive canine faecal samples from Croatia were used for DNA extraction and multilocus sequence typing with nested PCRs targeting five different gene loci: SSU rRNA, ITS1-5.8S-ITS2, beta giardin (bg), glutamate dehydrogenase (gdh) and triosephosphate isomerase (tpi). One third (33.7%) of the samples tested positive for *Giardia* antigen in the coproantigen ELISA. Shelter dogs were infected more frequently than household dogs (57.2 vs. 29.7%, $p < 0.01$). Amplification was obtained in 82.0, 12.8, 11.3, 1.5, and 31.6%, of the investigated samples at the SSU rRNA, bg, gdh and tpi loci and the ITS1-5.8S-ITS2 region, respectively. The dog-specific assemblages C and D were identified in 50 and 68 samples, respectively. The results demonstrate that *G. duodenalis* should be considered as a common parasite in dogs from South Eastern Europe. However, there was no evidence for zoonotic *Giardia* assemblages in the investigated canine subpopulation.

№11

Georgieva, D., V. Koinarski, P. Prelezov, Z. Kirkova and A. Ivanov, 2000. Role of wild carnivores in the epizootology and epidemiology of trichinellosis. *Bulgarian Journal of Veterinary Medicine*, 3, 4, 199-204.

The occurrence of trichinellosis in wild carnivores and their role in the epizootology and epidemiology of the disease was studied. In the period 1995-2000, 78 foxes, 45 jackals, 18 wolves, 21 martens, 16 wild cats, 6 badgers and 2 otters, shot in different regions of the mountains East Stara Planina and Sredna Gora, were necropsized. Twenty-four pieces from *crura diaphragmatica* obtained from each animal, were studied through trichinelloscopy.

The highest extensity of invasion (EI) was found out in martens. From all 21 martens, 13 (61.9%) were invaded by trichinellae. The positive results for the other wild animal species were as followed: 42.3% for foxes; 33.3% for jackals; 22.2% for wolves, 31.3% for wild cats and 33.3% for badgers. No trichinellae were present in the muscle samples taken from otters.

Those results show that the trichinellosis agent is circulating in nature among various wild carnivore species. They play an important role in the ecology of trichinellosis as reservoir of the infection and source for its transfer in synantropic foci.

№12

Georgieva, D., Z. Kirkova and A. Ivanov, 2001. A study on the incidence and diagnostics of dirofilariosis (heartworm disease) in carnivores. *Bulgarian Journal of Veterinary Medicine*, 4, 4, 231-236.

During the period 1997–1999, 258 canine blood samples were analysed. The animals were of a various origin: 65 working dogs, 27 shepherd dogs, 55 rural dogs, 40 stray dogs and 71 companion dogs. Microfilariae of *Dirofilaria immitis* were detected in 19 or 7.4% of all studied samples by the method of Knott. The incidence of parasites was the greatest in stray (12.5%) and rural (10.9%) followed by working (7.7%) and shepherd (7.4%) dogs. Dogs kept as companion animals were the least invaded (1.4%).

All 19 microfilaria-positive samples reacted positively with the Pet Check HTWMPT test (Idexx) for detection of *D. immitis* antigens.

The study on 25 blood samples from microfilariaemic dogs, showing doubtful clinical signs of dirofilariosis yielded positive results in 2 animals (8%) with the Pet Check test.

The uncomplete helminthological necropsy of 40 stray dogs, 78 foxes, 45 jackals, 18 wolves, 21 martens, 16 wild cats and 6 badgers revealed mature *D. immitis* organisms in the heart and pulmonary arteries of 5 stray dogs, 4 foxes, 2 jackals and 1 wolf.

The *D. immitis* invasion within the risk groups of stray, working and rural dogs and wild canids creates prerequisites for its transmission through mosquitos to other, less endangered, but susceptible canine populations.

Индуцирана заболяемост при децата. Паразитологично проучване на детските площадки в гр. Стара Загора

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ВЪВЕДЕНИЕ

Лавинообразното увеличаване броя на скитащите кучета у нас поражда редица проблеми, рефлектиращи върху човешкото здраве. Особено застрашени са децата. Те стават жертва както на непосредствената кучешка агресивност, така и на паразитите, с които кучетата контаминират околната среда.

Почвата е основен компонент от биосферата за човека, животните и растенията. Когато тя е контаминирана с паразитни елементи - яйца, ларви или цисти на паразити, съществува риск за възникване на епидемични и эпизоотични паразитози (Georgieva, D. et al, 1999). Този риск е особено висок за децата, играещи в пясъчните на детските градини и детските площадки. Там децата могат да се заразят с токсокароза, ехинококоза, токсоплазмоза, дипилидиоза, дифилариатоза и гр. зооантропонозни паразитози, защото бездомни котки и кучета имат широк достъп до тези пясъчници и ги контаминират с яйцата на паразитите (Despontier 2003; Fisher, 2003).

Целта на нашето изследване е да проучим контаминираността със зоопаразитни елементи на пясъчните и детските градинки в гр. Стара Загора и да изясним тяхното значение като фактор за инвазирането на децата.

МАТЕРИАЛ И МЕТОДИ

Паразитологично проучени са 38 обекта - детски площадки и детски заведения (градини и ясли) на територията на гр. Стара Загора през м. януари, 2005 г. Обектите се намират в централната градска част, жилищните комплекси „Три чучура“, „Казански“, „Чайка“, „Алана“, парк „Пети октомври“ и „Аязмото“ (Фиг.1).

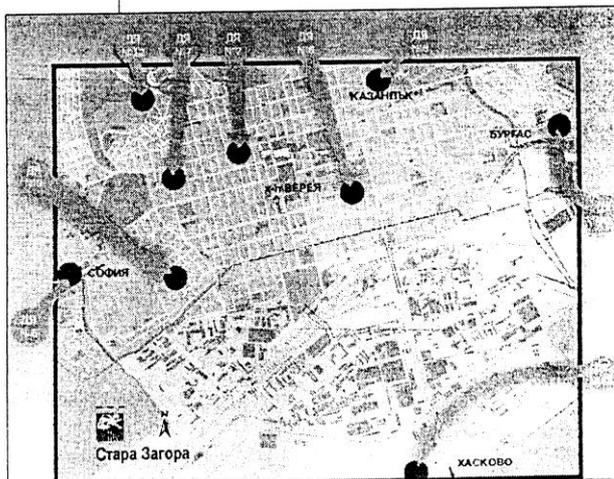
Материалът е пясък, събран от пясъчните на площадките за игра на децата и почва от градинките, оформящи кътовете за отдих, както и тези в детските ясли и градини - общинска собственост и едно частно детско заведение.

Използван е центрофужно-флотационният обогатителен метод на Data (Data, 1979).

Количеството изследван материал от всяка проба е 5 g, а флотиращ разтвор е Zn SO₄.

РЕЗУЛТАТИ И ОБСЪЖДАНЕ

В 30 от 38 (78.95%) обекта на обследване се установи наличие на паразитни елементи. Осем от обектите не бяха контаминирани, а в 3 са контаминирани и пясъчните и пръстта в градинките (табл. 1).



Фиг.1. Разпределение на детските ясли в Стара Загора по квартали

Kirkova, Z., D. Georgieva, E. Raychev, 2006. Study on the prevalence of trichurosis in different categories of dogs and wild carnivores. *Bulgarian Journal of Veterinary Medicine*, 9, №2, 141-147

In the period between 2003 and 2005, the prevalence of trichurosis in various categories of dogs and wild carnivores was studied. To this end, 508 coprological samples from dogs aged from 6 months to 14 years from different populated areas in North-east, Central, and South Bulgaria were studied using the flotation method of Fuleborn. Partial helminthological autopsies of the large intestine were performed in 113 foxes and 56 jackals. *Trichuris vulpis* infection was found in all studied categories of dogs. The highest extensity of infection was observed in hunting dogs (30%), followed by dogs used as guards of village yards (21.8%), dogs bred in kennels (9%), and home pets (6%). In the studied wild carnivores, *T. vulpis* infestation was found in 30.7% of the jackals, and in 12.2% of the foxes. The results showed that trichurosis is one of the commonest intestinal helminthoses in dogs and jackals.



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Original Contribution

**ПРОУЧВАНЕ ОПАРАЗИТЕНОСТТА С *LAMBLIA INTESTINALIS* СРЕД НЯКОИ
 РИСКОВИ ГРУПИ ХОРА**

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ABSTRACT

Giardia is the most frequently identified intestinal parasite worldwide.

Aim: The aim of this study was to relate personal data, socio-cultural and environmental characteristics for sporadic infection with *Giardia intestinalis*.

Patients and Methods: Prospective data collection was performed during the year 2004-2007. Stool samples from 738 children and adults were analyzed for *Giardia* cysts. There were determinate five risk groups: farmers family, children attending day care centers, adults in social care centre living in closed communities, children, living in gypsy communities and nurses in day care centers and hospitals. Each subject was interviewed and questionnaire completed. There 25 questions relating: age, sex, place of residence, profession, swallowing water while swimming, vegetable consummation, animals, at home, history of diarrhea and parasitic and viral intestinal infections, socio-economic status etc.

Results: Of the surveyed population n=738, 41% (5.6%) were infected. The frequency infection according to sex – 58% (42%) of males (females). *Giardia*-cysts was detected in children in age 0-15 years. A big percent of *Giardia* positive patients have lived in rural areas in village - 63%. Animals at home have 77.8% of patients. Drinking treated tap water 34.1% of patients.

Conclusion: Analysis revealed that age, place of residence, profession, animals at home, sources of irrigating of vegetables, density of living were factors, which indicate the importance of these factors in rural communities.

Key words: *Giardia intestinalis*, sporadic Giardiasis, risk groups, risk factors

№16

Kirkova, Z., E. Raychev, D. Georgieva. Studies on Feeding Habits and Parasitological Status of Red Fox, Golden Jackal, Wild Cat and Stone Marten from Sredna Gora, Bulgaria. *Journal of life sciences*, 5, 4, 264-270, 2011.

In order to define the role of wild carnivores in the epidemiology of parasitoses with veterinary and medical importance their parasitological status and feeding habits were studied. In the period 2001-2006 the feeding habits of 167 foxes, 78 jackals, 40 wild cats and 23 stone martens from the area of Sredna Gora, Bulgaria were investigated. 113 of the foxes, 56 of the jackals, 22 of the wild cats and 21 of the martens were subjected to helminthological study. 147 wild boars and 26 badgers from the same area were subjected to trichinelloscopy. Rodents were the main food of the wild cats (82.7%), martens (52%) and foxes (50%). The main food of the jackals was carrion from domestic and wild animals (79.5%). 95.5% of the foxes, 100% of the jackals, 95.5% of the wild cats and 89% of the stone martens were infected with one or more helminth species. The prevalence of the most important helminths: *Trichinella* spp., *Taenia* spp. and *Ancylostoma* spp. was high in all carnivores examined. In the infected with *Trichinella* spp. animals only *T. britovi* was demonstrated. The wild boars and badgers were not infected with *Trichinella* spp. The correlation between the feeding habits and parasitological status is discussed.

№17

Prelezov P., Z. Kirkova, C. Bauer, D. Georgieva, V. Koinarski, A. Ivanov. Survey on the anthelmintic efficacy of benzimidazoles against gastrointestinal strongylids of sheep in southeastern Bulgaria. *Trakia journal of sciences*, 6-1, 82-86, 2008

The efficacy of benzimidazole anthelmintics was investigated in 15 randomly selected sheep farms located in southeastern part of Bulgaria. The in vitro egg hatch assay (EHA) was performed after anaerobical storage of mixed faecal samples from each farm for 2 – 5 days. Pure thiabendazole was used in solutions of 0.02; 0.04; 0.05; 0.08 and 0.1 µg.ml⁻¹. Effective dose (ED 50) was calculated. On each farm, 30 sheep were allocated at random into two groups of 15 animals each: an untreated control group and a group that was orally administrated albendazole (Valbazen®, Pfizer Animal Health) (3.8 mg.kg⁻¹) or oxfendazole (Oxfenil®, Virbac Animal Health) (5.0 mg.kg⁻¹). Individual faecal egg counts were estimated before and 14 days after treatment and faecal egg count reduction (FEER) was calculated, for performing the in vivo egg faecal count reduction test (FECRT). Mixed farm faecal samples were cultivated for 7 days at 27°C for developing and identification of strongylid larvae III. The predominant genus was *Haemonchus*. In all the examined sheep farms the ED50 of thiabendazole was below 0.1. All the flocks showed FEER values over 95%. No developed anthelmintic resistance was detected. From the results of the present trial can be concluded that benzimidazoles are high effective against sheep gastrointestinal strongylids in southeastern Bulgaria.

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ПРОУЧВАНИЯ ВЪРХУ ЕТИОЛОГИЯТА И НЯКОИ
ЕПИДЕМИОЛОГИЧНИ АСПЕКТИ НА СТОМАШНО-
ЧРЕВНИТЕ СТРОНГИЛИДОЗИ ПО ДРЕБНИТЕ
ПРЕЖИВНИ ЖИВОТНИ

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Summary

Prelezov, P., A. Ivanov, V. Koinarski, P. Iliev & Z. Kirkova, 2013. Studies on the etiology and some epidemiological aspects of gastrointestinal strongylidoses of small ruminants. *Bulg. J. Vet. Med.*, **16**, Suppl. 1, 156–163.

Gastrointestinal strongylids (type Nematoda, order Strongylida) constitute the major part of helminthic fauna of small ruminants causing serious economic losses at a global scale. They are responsible for a dramatic reduction in production traits and even for death of animals. That is why the present study aimed to investigate the diversity, distribution, and the level of infestation of gastrointestinal strongylids on small ruminants in Southern Bulgaria. Sixty one sheep and eleven goat herds were examined by the modified McMaster's technique and cultivation of larvae. Infection was established in 87% of sheep herds and 91% of goat herds with mean intensity of invasion between 200 ± 12.8 and 655 ± 43.2 . Six genera of gastrointestinal strongylids were observed: *Haemonchus*, *Chabertia/Oesophagostomum*, *Ostertagia*, *Nematodirus* and *Trichostrongylus*.

Key words: diversity, gastrointestinal strongylids, small ruminants

ЛЕВАМИЗОЛОВА РЕЗИСТЕНТНОСТ ПРИ СТОМАШНО- ЧРЕВНИТЕ СТРОНГИЛИДИ ПО ОВЦЕТЕ В БЪЛГАРИЯ

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Summary

Iliev, P., P. Prelezov, A. Ivanov & Z. Kirkova, 2013. Levamisole resistance in gastrointestinal strongylids on sheep in Bulgaria. *Bulg. J. Vet. Med.*, 16, Suppl. 1, 164–170.

Levamisole is one of the first anthelmintic in Bulgaria and is still widely used. Its frequent and not always proper application is a primary cause for reduction of its efficacy to critical levels. To establish the presence of levamisole resistance among gastrointestinal strongylids (GIS), we used the following *in vitro* tests: Larval development assay (LDA), Egg hatch paralysis assay (EHPA), and Larval migration inhibition test (LMIT). The effective doses required to inhibit 50% of the larvae (ED_{50}) were: for LDA from 0.198 to 1.785 μM ; for EHPA from 0.095 to 13.274 μM ; for LMIT from 0.140 to 2.812 μM . Based on world references we assumed that ED_{50} values of levamisole showing presence of resistance are over 1.56 μM for LDA, 2.45 μM for EHPA and 1.2 μM for LMIT. On the basis of results, in one of the 12 examined sheep herds, levamisole resistance was detected with ED_{50} values: in LDA – 1.786 μM ; in EHPA – 13.274 μM and in LMIT – 2.812 μM .

Key words: egg hatch paralysis assay, *in vitro*, larval development assay, larval migration inhibition test, levamisole resistance, sheep, strongylids

№20

Iliev P., Prelezov P., A. Ivanov, Z. Kirkova. Investigation on the benzimidazole resistance in gastrointestinal strongylids of sheep in southeastern Bulgaria. *Trakia journal of sciences*, 12-2, 189-192, 2014

Benzimidazoles are the most commonly used anthelmintic drugs for the chemical control of sheep strongylidoses in Bulgaria. The intensive and not always proper application could be able to cause a reduction of their efficacy. To establish benzimidazole resistances among gastrointestinal strongylids, two in vitro assays were performed – Egg Hatch Assay (EHA) and Larval Development Assay (LDA). The effective concentrations required to inhibit 50 % of egg hatching (egg dead - ED50) or larval development (larva dead - LD50) were: for EHA from 0.0114 to 0.2023 µg/ml and for LDA from 0.0017 to 0.5817 µM thiabendazole. All the data were analysed. Based on the reference we assumed that ED50 values showing a presence of resistance are over 0.1 µg/ml and LD50 - over 0.12 µM thiabendazole. According to the results discovered here, in two of 13 sheep flocks examined, a benzimidazole resistance was detected with values of ED50 between 0.1132 and 0.2023 µg/ml and for LD50 between 0.2940 and 0.5817 µM thiabendazole.

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AN UPDATE ON DIROFILARIOSIS IN DOGS AND WILD CANINES IN BULGARIA

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SUMMARY

A study on the prevalence of the dirofilariosis in dogs and wild carnivores in Bulgaria was performed from 2001 to 2007. Blood samples from 511 dogs out of heartworm prophylaxis originating from different parts of the country were tested. Microfilariemia was found in 52 (10.17 %) of the samples by the modified Knott's test. *Dirofilaria immitis* larvae were found in 47 (9.19 %) of the samples and *Dirofilaria repens* larvae in 7 (1.36 %). Two (0.4 %) of the dogs were found to be positive for both *D. immitis* and *D. repens* larvae. The samples positive for *D. immitis* larvae, were antigen positive by Pet Check HTWMPT test (IDEXX). The samples containing only *D. repens* larvae were antigen negative by Pet Check. Three (6.6 %) of 45 microfilaria negative samples were antigen positive. Infected animals were found in all parts of the country.

The necropsy of 113 foxes, 56 jackals, 22 wild cats, 21 martens and 2 wolves revealed *Dirofilaria immitis* infection in 4 (3 %) of the foxes and 5 (8.9 %) of the jackals. The intensity of the infection was from 2 to 16 specimens.

Our results show that the wild canines are natural reservoir and source of infection for dogs. The trend to increasing of the prevalence of dirofilariosis in dogs, found in comparison with previous studies, suggest that heartworm prophylaxis should be considered in Bulgaria.

Key words: *Dirofilaria immitis*, *Dirofilaria repens*, zoonoses, helminthes, dogs, wild canides.

ПРЕВАЛЕНТНОСТ И ТИП НА ИНВАЗИЯТА С МАЛОФАГИ (INSECTA: PHTHIRAPTERA) ПО ГЪЛЪБИТЕ (*COLUMBA LIVIA*) В ЮГОИЗТОЧНАТА ЧАСТ НА БЪЛГАРИЯ

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РЕЗЮМЕ

При изследване на 228 гълъби от 15 населени места на Югоизточна България са доказани 4 вида хапеци въшки: *Columbicola columbae*, *Goniocotes bidentatus*, *Neocolpocephalum turbinatum* и *Hohorstiella lata*. Установени са общата и видовата превалянтност на малофагите по изследваните гълъби. Опаразитени с един или няколко вида са били 209 птици (91,7 %). Сред отделните видове малофаги, най-висока превалянтност е наблюдавана при вида *C. columbae* – 91,7 % (209 от изследваните гълъби), следван от *G. bidentatus* – 39,9 % (91 птици), *N. turbinatum* – 7,9 % (18 птици) и най-ниска е превалянтността на *H. lata* – 3,5 % (8 птици).

Изследван е типът на инвазия с малофаги по домашния гълъб. При 162 (77,5 %) от инвазираните гълъби е установена моноинвазия. При останалите 47 (22,5 %) птици инвазията е била от смесен тип. От тях 40 (19,2 %) са били инвазирани с 2 вида, 4 (1,9 %) – с три вида и 3 (1,4 %) – с 4 вида.

Ключови думи: Pthiraptera, Mallophaga, хапеци въшки, гълъби, превалянтност.

ВЪЗМОЖНИ ЗООНОЗНИ АСПЕКТИ НА ГИАРДИАЗАТА ПРИ ЧОВЕКА

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РЕЗЮМЕ

Гиардиазата е космополитна паразитоза. В България сред хората е регистрирано носителство под 1 %. Заболяването е разпространено и сред животните, но няма данни за разпространението му у нас. За да проучим някои възможни зоонозни аспекти на гиардиазата при човека, проведохме анкета сред 40 лица, опаразитени с *G. intestinalis* относно условията, в които отглеждат домашните си любимци. Изследвахме чрез бърз имунохроматографски тест 40 животни – 25 кучета и 15 котки. Установихме, че 80 % от тях осъществяват контакти с други животни, рискови за опаразитяване; 67,5 % не са профилактирани никога, а при болест само 35,5 % са преглеждани и лекувани от ветеринарен лекар. Опаразитени с *G. intestinalis* са 3 (7,5 %) животни.

Ключови думи: гиардиаза, зоонозни аспекти, имунохроматографски тест.

ПРОУЧВАНЕ ЕКСТЕНЗИТЕТА НА ИНВАЗИЯ С *GIARDIA INTESTINALIS* ПРИ ДОМАШНИ КУЧЕТА И КОТКИ С ДИАРИЧЕН СИНДРОМ

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РЕЗЮМЕ

През периода от 2006 до 2011 г. в секция „Паразитология“ на ВМФ, Тракийски университет беше проведено проучване върху екстензитета на инвазия с *Giardia intestinalis* (Sarcostomatophora, Hexamitidae) при домашно отглеждани кучета и котки с диаричен синдром. Изследвани бяха 100 бр. фекални проби от кучета и 29 бр. от котки, чрез нативен препарат и антигенен тест RIDA Quick® Giardia (R-Biopharm AG, Germany). Тестът беше позитивен при 18 % от кучетата и при 20,7 % от котките, докато при нативното микроскопско изследване цисти на *Giardia* бяха открити в 7 % от кучетата и в 6,9 % от котките.

Резултатите от изследванията показаха, че гиардиозата е съществен фактор в етиологията на диаричния синдром при младите кучета и котки.

Ключови думи: Giardiasis, prevalence, dogs, cats.

Dirofilariasis in dogs and wild carnivores in Bulgaria

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A study of the prevalence of the dirofilariasis in dogs and wild carnivores in Bulgaria was conducted from 2001 to 2006. Blood samples from 487 dogs out of heartworm prophylaxis originating from different parts of the country were tested. Microfilariaemia was found in 42 (8.62%) of the samples by the modified Knott's test. Infected animals were found in all parts of the country.

All microfilaria positive samples were antigen positive by Pet Check HTWMPT test (IDEXX). Three (6.6%) of 45 microfilaria negative

samples were antigen positive.

The necropsy of 113 foxes, 56 jackals, 22 wild cats and 21 martens revealed *Dirofilaria immitis* infection in 4 (3%) of the foxes and 5 (8.9%) of the jackals. The intensity of the infection was from 2 to 16 specimens.

Our results show that wild canides are a natural reservoir and source of infection for dogs. The trend of increasing prevalence of dirofilariasis in dogs, when compared to previous studies, suggests that heartworm prophylaxis should be considered in Bulgaria.

Determination of Canine Immune Deficits which lead to Disseminated *Leishmania infantum* Infection in American Foxhound Dogs

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Abstract

Leishmaniasis is a vector-borne disease caused by the protozoa *Leishmania*. Infection with *L. infantum* causes fatal visceral leishmaniasis in dogs globally. After 2000 Leishmaniasis was found to be endemic in Foxhounds within United States. Since then numerous Foxhounds have tested positive, leading to an average 5% seropositivity in all Foxhound hunts.

There is concern for spread of *Leishmania* to other dogs and humans, as presence of infected dogs is the primary risk factor for disease in endemic locations. Vector transmission has not been identified in the U.S.; instead we have identified a primary means of transmission to be vertical from bitch to pup. This unique means of infection is likely to predispose Foxhounds vs. other breeds to infection and leads to an inability of infected dogs to mount a productive adaptive immune response due to loss of *L. infantum*-specific T and B cells during development. Importantly, vertical transmission may make serology, the most common method of testing for this disease, quite inappropriate as a stand-alone test for the current strain of Leishmaniasis in the U.S.. We have developed a highly sensitive and specific quantitative real-time PCR (qPCR) based assay to detect *L. infantum* infection in canine blood prior to seroconversion. This assay is based on amplification of parasite kinetoplast DNA.

This disease provides a canine model system to analyze cell-mediated immunity to vertically transmitted pathogens and highlights that serology may be grossly underestimating actual disease prevalence of canine Leishmaniasis in this country. Our studies are aimed to determine whether presence of parasites during gestation alters the ability of the canine immune system to mount a productive immune response to *L. infantum*, predisposing foxhounds to this disease.

SURVEY ON ECTOPARASITES OF DOGS (*CANIS FAMILIARIS*) IN BULGARIA

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During 2012, ectoparasites obtained by body search, total body comb, and deep ear swabs were collected from 295 dogs (166 male, 129 female; approximately 2 months to 15 years old; 263 privately owned, 32 from shelter) originating from all regions of Bulgaria (Sofia Valley, Danube Plain, Upper Trakia Valley, Kazanlak Valley and Black Sea region). Privately owned dogs were used as guards, shepherds, or for hunting or breeding in kennels. Ear swabs were examined for the presence of ear mites, and skin scrapings were collected if clinical signs indicated the presence of other mite infestations.

Overall, 63.1% of the dogs tested positive for ectoparasites. Prevalence rates for ectoparasites were 48.1% for fleas (*Ctenocephalides canis* 44.1%, *Pulex irritans* 15.9%, *C. felis* 4.1%, *Archaeopsylla erinacei* 0.3%), 23.7% for ticks (*Rhipicephalus sanguineus* 19.7%, *Ixodes ricinus* 6.4%), and 0.3% for *Cheyletiella* spp.. No ear mites were found in any of the swab samples.

There was no difference between sexes for the prevalence of any of the parasites recovered. However, prevalence of infestation with *P. irritans* was significantly ($p < 0.0001$) higher in shelter dogs than in privately owned dogs. In addition, the intensity of infestation of this species was higher in shelter dogs. In contrast, intensity of infestation of *C. canis* was higher ($p = 0.048$) in privately owned dogs than in dogs from shelters.

Mixed infestations with up to four ectoparasite species were recorded in 34.9% of positive dogs. Most common combinations were *C. canis* + *P. irritans* (37 dogs, 19.9% of positive dogs) and *C. canis* + *R. sanguineus* (16 dogs, 8.6% of positive dogs). This survey revealed a generally high prevalence of ectoparasites in dogs in Bulgaria. The data indicate that measures should be taken to reduce the rate of infestation of these parasites, including raising awareness, employing effective treatment, and improving local animal care.

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FECAL EXAMINATIONS OF DOGS (*CANIS FAMILIARIS*) IN BULGARIA

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During 2012, fecal samples were obtained from 294 dogs (165 male, 129 female; approximately 2 months to 15 years old; 69 ≤12 months, 252 >12 months; 262 privately owned, 32 from an animal shelter) originating from all regions of Bulgaria. Privately owned dogs were used as guard dogs, shepherds, or for hunting or breeding in kennels. Fecal samples were examined for helminth and protozoan infections using a modified McMaster method and the larval migration technique according to Baermann. A subset of 148 samples was subjected to a standard ELISA technique for the detection of *Giardia* coproantigen.

The overall frequency of helminth infections in dogs was 52.0% (95% CI 46.3–57.7%). The most frequently diagnosed faecal forms were those of hookworms (37.8%) and *Trichuris* (24.1%), followed by ascarids (7.8%; *Toxocara* 6.8%, *Toxascaris* 1.0%), *Capillaria* (6.8%), cestodes (1.3%; taeniid 1.0%, dipylidiid 0.3%), coccidians (1.0%), and pentastomid eggs (0.7%). All samples were negative for lungworm larvae. The *Giardia* ELISA revealed positive results for 33.8% of samples examined. Single and multiple infections with up to four parasites concurrently were found in 31.0% and 21.1% of the fecal samples, respectively.

Within the group of infected animals, mixed endoparasite infections were recorded more frequently in dogs older than one year compared to younger animals (45.5% vs. 26.8%; $p = 0.042$). Young dogs (≤12 months) were parasitized more frequently with *Toxocara* (18.8% vs. 3.1%; $p < 0.0001$) and *Giardia* (65.0% vs. 22.2%; $p < 0.0001$) than the older dogs. Hookworm infections were more frequently diagnosed in male dogs than in female dogs (44.2% vs. 29.5%; $p = 0.011$).

By providing basic data on the current situation regarding canine helminth and protozoan infections, the results of this survey emphasize the need to attend to parasites of dogs from the veterinary point of view with respect to both, appropriate diagnostics and control.

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**РЪКОВОДСТВО
ЗА УПРАЖНЕНИЯ
ПО ВЕТЕРИНАРНА
ПАЗИТОЛОГИЯ**