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**The XXIIIth Congress
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ABSTRACTS

SESSION VI

**Current trends in medical and veterinary
parasitology**

May parasitic invasions have a negative impact on sled dogs achievements?

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The achievements of sled dogs in races depend on their training and on perfect health. Parasitic invasions, including intestinal parasites or vector-borne infections, may lead to anaemia, and may affect joints, the heart or even cause death. Between December 2009 and October 2010, one hundred and twenty-six individual fecal and blood samples were collected from 26 sled dog kennels situated in different regions of Poland. The prevalence of intestinal parasites (helminths, *Cryptosporidium*, *Giardia*) was determined by salt flotation or IFA test. The prevalence of vector-borne infections (*Anaplasma phagocytophilum*, *Babesia canis*, *Hepatozoon canis*, *Dirofilaria repens*) was determined using PCR/nested PCR. Additionally, 25 serum samples originating from 3 kennels (Mazovia province) were tested for antibodies against tick-borne encephalitis virus (TBEV).

Overall the prevalence of intestinal parasites was 68% (73/108). In 51 samples, a single species of nematode was detected (47%), two species were detected in 13% of samples, three species in two dogs. The most prevalent were the hookworms *Uncinaria/Ancylostoma* identified in 36% of kennels and in 34% of dogs. *Toxocara* sp. eggs were detected in 36% of kennels, in 17% of dogs. *Trichuris* sp. eggs were found in 20% of kennels (5/25), and in 13% of dogs. (Oo)cysts of intestinal protozoa were detected in 31% of sled dogs (*Giardia* infection – in 54% of kennels [13/24], in 28% of dogs; *Cryptosporidium* infections – in 37.5% of kennels [9/24], in 13% of dogs).

Babesia canis DNA was detected in 11 sled dogs (4 with clinical babesiosis, 7 asymptomatic; 8.7%). Three serum samples originating from one kennel tested positive for TBEV antibodies (total seroprevalence: 3/25=12%, local seroprevalence: 3/12=25%). *Dirofilaria repens* DNA was detected in 6 dogs (23.1%) from two sled dog kennels situated near Grodzisk Mazowiecki. No blood samples tested positive for *A. phagocytophilum* or *H. canis* DNA.

The present study has demonstrated a low prevalence of vector-borne pathogens in comparison to intestinal parasites in working sled dogs during the racing season, as well as the need to monitor the *D. repens* invasion. It has also confirmed the role of reservoir host surveillance in the epidemiology of TBE.

The study was partially funded by National Science Centre (NCN) grant OPUS 2011/03/B/NZ802212.

Biodiversity of the strongylids nematodes (Strongylidae) in horses from a stud farm

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The aim of the study was to determine the biodiversity of the strongylid nematodes (Strongylidae) in horses from a large stable. Also, a possible relationship between the presence of species of the strongylid community and the age of the host, pasture season as well as procedure of deworming (time of treatment, type of antiparasitic drug, resistance of nematodes) were examined. Faecal samples weighing about 500 grams were collected from 18 horses, 24 hours after treatment with ivermectin. The obtained samples were checked and any nematodes found were preserved in 70% ethyl alcohol with glycerin and identified to the species. Among 14,875 strongylids isolated from the fecal samples 16 species from 3 genera of the cyathostomin subfamily (Cyathostominae) and 1 species from the strongylin subfamily (Strongylinae) were found. Among the Strongylidae the most prevalent species were *Cyathostomum catinatum* (100%), *Cylicostephanus longibursatus* (94%), *Cylicostephanus minutus* (89%), *Cylicocyclus nassatus* (89%), however the relative abundance was the highest for *Cylicocyclus nassatus* (42.1%), *Cylicostephanus longibursatus* (23.7%), *Cyathostomum catinatum* (11.6%), *Cylicocyclus leptostomum* (5.1%) and *Cyathostomum pateratum* (4.4%). The number of species in one horse was ranging from 3 to 15.

Preliminary study on the vertical transmission of *Babesia microti* in BALB/c mice

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Babesia spp. (Apicomplexa, Piroplasmida) are obligate parasites of many species of mammals, causing babesiosis, a malaria-like infection. Three routes of infection are recognized. The main route is by tick bite. Parasites can also be transferred via blood transfusion or direct contact with the blood of infected hosts. The third, vertical route of infection is poorly recognized and understood. Transmission of *Babesia* between mother and offspring was discovered in different mammals: bovines, sheep, dogs, horses and laboratory rats. There are also a few reports on congenital infections of *B. microti* in humans.

Our study was focused on vertical transmission of *B. microti* under well-controlled laboratory conditions. The aim was to assess the risk of occurrence of congenital infection in BALB/c mice with acute and chronic infection of *B. microti*.

Six groups of mice (four experimental and two control) were used throughout this study. In experimental groups, females were mated on the 1st day of *Babesia* infection (Group **G0**), (2) on the 28th day post infection (dpi) (**G28**), in post-acute phase of parasite infection, and on 90 and 150 dpi (**G90** and **G150** group, respectively), in the chronic phase of parasite infection. Control groups consisted of mice infected with *B. microti* (KBab group) and of mice not infected with any parasite (K group).

Births were reported in dams from three experimental groups. Litters were obtained from 50% of the females (3/6) infected in post-acute phase (G28) and 33% females (2/6) in both the G90 and G150 groups. No offspring resulted from dams mated in the pre-acute phase of infection (G0). Congenital *B. microti* infections were detected by means of PCR in all pups in the litters in the G28, G90, G150 experimental groups. Prevalence on the basis of microscopical observation was 52% (22/42). Parasitaemia varied between 0.01–0.001%. The vertical route of infection of *B. microti* was confirmed for the first time in BALB/c mice.

The seroprevalence of antibodies against Spotted Fever Group rickettsiae among forestry workers from northern Poland

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The prevalence of IgG antibodies to spotted fever group rickettsiae (SFGR) was investigated by the indirect enzyme immunoassay (Fuller Laboratories, CA, USA) in a sample of 485 forestry employees of the Kujawsko-Pomorskie province (northern Poland). Sera were collected in 2005 from 89 females (mean age 43.7 years) and 396 males (mean age 43.5 years) working in 9 different forest managements and the Regional Directorate of the State Forest in Toruń, and were kept frozen until testing.

Overall, as many as 198 (45.7%) persons were found to have *Rickettsia* spp. antibodies, with a significantly higher prevalence in males (45.7%) than females (19.1%). The results show that both groups were clearly being exposed to SFGR. Moreover, 50% of the foresters and other workers with outdoor activity were positive in comparison to 21.3% of the office workers and other workers with indoor activity. The time of employment had no significant influence on seroprevalence in either population, although the highest prevalence was noted among persons working more than 30 years (60.1%). Age-specific prevalence rates for the ≥ 20 , 21–30, 31–40, 41–50, 51–60 and over 60 years old groups were 55.5, 29.8, 41.8, 32.5, 42.1 and 52.9%, respectively.

The difference in seroprevalence between those who declared no tick bites and the tick-bitten population was observed (38.2% vs. 50.7%).

The results indicate that spotted fever is endemic in the region studied.

Further investigations to determine species-specific antibodies (*R. helvetica*, *R. sibirica*, *R. slovaca*) using indirect immunofluorescence assay (MIF) (Fuller Laboratories, CA, USA) are being conducted.

Kampinoski National Park – a risk area for Spotted Fever Group rickettsioses?

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Spotted fever group (SFG) rickettsiae are mainly associated with hard ticks (Ixodidae). At least 13 species are known to be pathogenic for humans who, being their accidental hosts, acquire infection by a tick bite.

Kampinos National Park (KNP) is the largest natural area in Poland. It borders on Warsaw, the Polish capital city and, as a natural recreational hinterland, is frequently visited by its inhabitants and tourists.

The aim of our study was to detect and identify SFG rickettsiae in ticks from the KNP and estimate their infection level with this pathogenic bacteria.

Altogether 550 adult ticks: 263 *Ixodes ricinus* and 287 *Dermacentor reticulatus* were collected in April/May 2011 from vegetation in 12 different sites in forested and grassy areas. A total DNA was extracted by lysis of crushed ticks in NH₄OH. Then, a semi-nested PCR was performed using three genus specific primers. Of them, Ric and Ric U8 yielded a 1.385 bp fragment encompassing almost the complete 16S rRNA gene, while Ric and Ric RT yielded a 757 bp fragment.

The DNA of *Rickettsia* spp. was found in 51 (19.4%) *I. ricinus* and 34 (11.8%) *D. reticulatus*. Then, all 84 positive samples were tested with SLO1F/SLO1R (outer) and SLO2F/SLO2R (inner) (355 bp) primers targeting a specific fragment of rOmpA-encoding gene, and none of *I. ricinus* and all 34 *D. reticulatus* were positive.

Sequences of 16S rRNA gene fragment from 10 *I. ricinus* showed 100% homology to *R. helvetica* while these from 10 *D. reticulatus* were identical to the *R. raoulti*, Marne strain. Moreover, the sequences of amplicons with primers specific for the *OmpA* gene also showed 100% similarity with *R. raoulti*. So far no *R. slovaca* has been detected.

Though the pathogenicity of the *R. raoulti* has not yet been proven definitively, this species and *R. helvetica* should be added to the list of potentially dangerous pathogens transmitted by ticks in KPN. The obtained results provide a good starting point for epidemiological studies to come.

Estimation of parasitological contamination of municipal sewage sludges produced in Poland

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The agricultural use of sewage sludge can cause microbiological and parasitological contamination of soil, water systems and cultivated plants, and is a risk for human and animal health. For this reason there is a duty to monitor the safety of these substances, including the need for parasitological examination to detect the live eggs of parasites from the genera *Ascaris*, *Trichuris* and *Toxocara*. The Department of Parasitology and Invasive Diseases NVRI in Pulawy has elaborated a new very effective method for parasitological examination of sewage sludge. Depending on the type of parasite egg, its sensitivity is 5–8 times higher than the Quinn method and several dozen times more sensitive than the most popular method, according to the PN-Z-19000-4 standard.

Investigations were carried out on dehydrated sewage sludge from 92 municipal treatment plants in Poland (minimum 5 treatment plants from each province). Samples were obtained from 36 large treatment plants (Population Equivalents – PE >100 000), 38 from medium (PE 15 000–100 000), 9 smaller (PE 10 000–15 000) and 9 (PE 2 000–10 000). Samples were examined with the use of the method elaborated in the laboratory.

Eggs of *Ascaris*, *Trichuris* and *Toxocara* were found in 91 samples. The mean number of parasite eggs per 1 kg of dry weight (d.w.) equaled 10 343 and ranged from 447 to 319 878/kg d.w. *Ascaris* eggs were detected the most frequently (87 positive samples; on average 5 604 eggs/kg d.w.) and *Toxocara* eggs (88 positive samples; on average 3 664 eggs/kg d.w.). Less numerous were *Trichuris* eggs (57 positive samples; on average 1 074 eggs/kg d.w.). The greatest numbers of parasite eggs were contained in sewage sludge produced by the largest treatment plants (on average 15 206 eggs/kg d.w.) and the lowest in treatment plants with PE <15 000 (on average 3 233 and 3 453 eggs/kg d.w.). Such disproportions were particularly visible in the case of *Ascaris* eggs, which numbered 9 750 per 1kg of d.w. in treatment plants with PE > 100 000, 4157 in those with PE 15 000–100 000, 430 for PE 10 000–15 000, and 311 for PE 2 000–10 000.

The obtained data shows that intestinal parasite eggs occur commonly in sewage sludge produced by treatment plants in Poland. It must be stressed that the number of live eggs in examined samples exceeded the acceptable levels described in the regulations of the Polish Ministry of Agriculture and Ministry of Environment by many times.

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The occurrence of pathogenic free-living amoeba in city's fountains and sandboxes

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Free-living amoebae from genus *Acanthamoeba* are widely distributed in the environment. They can be found in water, soil, sand, and air. Some isolates can be a threat to human health. They can cause *Acanthamoeba keratitis* (AK), granulomatous amoebic encephalitis (GAE), and tissue or skin diseases.

The aim of this study was to establish the presence of free-living *Acanthamoeba* in the Poznan city's fountains and sandboxes and to demonstrate that they can be a threat to human health.

The amoebae were isolated from fountains, their sprays and from wet sand in sandboxes in children's playgrounds. Samples were collected during the period from May to August 2012. The strains of amoebae were isolated from environmental samples by cultivation on non-nutrient agar. Water samples were collected (ca. 150 ml) and filtered through a 0.45- μ m pore size filter. Filters were inoculated on non-nutrient agar plates seeded with *Enterobacter aerogenes*. The pathogenicity tests of the isolates were carried out on experimental animals. The identification of the isolates was based on their morphology.

Our results confirm that free-living amoebae were identified in all examined fountains. Free-living amoebae potentially pathogenic to humans were isolated from four fountains and three water sprays. This study showed that the free-living amoebae from genus *Acanthamoeba* were present in all examined sandboxes. Pathogenic amoebae were isolated from 7 of 13 examined sandboxes. All isolated amoebae belonged to the genus *Acanthamoeba* spp.

The presence of pathogenic protozoans in Poznan city's fountains, sprays and sandboxes can be a potential source of danger for human health, especially for children.

***Trichinella* in wildlife in Carpathian region of Ukraine and Slovakia**

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The name *Trichinella* refers to the nematode worms of the genus *Trichinella* Railliet, 1895 which cause one of the most dangerous zoonotic diseases – trichinellosis. *Trichinella* infections have been reported for almost all mammal species as well as for reptiles and birds. These parasites can also reach a human through the consumption of raw or undercooked meat with larvae of *Trichinella*. In Ukraine, *Trichinella* infection has been detected in domestic pigs and humans, while in Slovakia, the parasite circulates in almost exclusively in the sylvatic cycle with occasional human outbreaks. Moreover, in both countries, especially in the Carpathian region, game animals play the key role in infecting humans. In the period of 2009–2012, muscle samples from potential host species were collected both in Ukraine and Slovakia with the aim of identifying the major hosts of sylvatic trichinellosis in the Carpathian region. Muscle samples from ungulates (20) and carnivores (141) from the Carpathians in Ukraine and 131 carnivore and 62 wild boar samples from the Carpathians in Slovakia were studied for the presence of *Trichinella* by standard methods. The parasite larvae isolated from infected animals were identified by multiplex PCR analyses (Borsuk et al., 2003; Pozio and La Rosa, 2003). *Trichinella* was found in all areas of the Carpathian region of Ukraine. Larvae were detected in 10.5% of investigated wild boars, 20% wolves, 26.2% red foxes, 13.6% martens, 20% badgers and lynx (2 infected from 2 investigated). Parasites were not found in samples from roe deers, perhaps because of the food preferences of these species. Correspondingly, wolves and foxes were found to be the main reservoir of *Trichinella* in the sylvatic cycle in the Carpathian region in Ukraine. In Slovakia, no infected wild boar was found within the study. *Trichinella* larvae were found in the muscles of 19% of foxes. In addition, 41.7% of rock martens, 44.4% of pine martens, 35.7% of polecats, one wolf, one otter, one brown bear, one lynx and one domestic cat were found to be infected. Our studies demonstrate the presence of a single species, *Trichinella britovi*, in the Carpathian region of both Ukraine and Slovakia, being found in all infected animals. No mixed infections were found. At the same time, *T. britovi* (found in the majority of infected wolves and red foxes) is a dominant *Trichinella* species in the sylvatic cycle throughout Ukraine and also in Slovakia. The extent of trichinellosis infestation in wild mammals is higher in the Carpathian regions of both countries when compared the average prevalence: 23% in the Carpathians compared to 15% in the whole of Ukraine, and 19 % in foxes from Carpathians compared to less than 10% throughout Slovakia. This is arguably caused by high densities of predator populations in mountain regions that correspondingly lead to increased levels of scavenging and cannibalism and also colder climate carcasses. All these factors increase the prevalence of *Trichinella* amongst wild animals in sylvatic cycles and constitute a risk of infection for animals in domestic cycles and for humans.

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Hirudotherapy – a new trend in veterinary medicine

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Leeches have been used in the treatment of certain diseases since ancient times. There are a few species of medicinal leeches. The adult form of the European medicinal leech *Hirudo medicinalis* (L. 1758) has a large posterior sucker, which is used in crawling, whereas the smaller sucker at the head encloses the mouth, which has three jaws. Secretions from the salivary glands of medical leeches contain more than 100 bioactive substances. Leeches supplied by commercial facilities usually belong to the Eastern phylogroup of *H. verbana*.

Hirudo medicinalis have a wide range of applications in the veterinary sphere. Among patients are dogs, cats and horses. The number of leeches used in the treatment vary per patient. Usually 1 leech is applied per 10 kg of body mass of an animal, while 5 to 15 leeches are used per animal for horses. A therapist places a leech at the site of disease. Mostly, the animals don't show immediate nervous reactions when bitten by a leech. *Hirudo medicinalis* must stay on the skin until satiety, which generally takes 30–60 minutes for dogs and cats and 40–120 minutes for horses (Henne, 2010). When the leech falls off, the resulting bleeding is part of the therapy. Such bleeding should not be inhibited, and in this way, 50 ml of blood is lost. After the cessation of bleeding, the therapist treats the area with bandages.

The most common indications for the use of leeches in dogs are hip dysplasia, elbow, arthritis, acute and chronic inflammatory conditions or injuries of the tendons, fascia or vertebrae (spondylosis, discopathies, cauda equina). In addition, hirudotherapy has been successfully used to treat inflammation of the nerves of different pathogenesis, hematomas and eczema (Henne, 2010). Incidentally, leeches are used in the treatment of post-surgical scars. In the treatment of horses, specific indications are ataxia with different backgrounds, plastic hoof disorders (especially in the form of acute laminitis), myositis, spondylosis and diseases of fascia and tendons. Absolute contraindications to hirudotherapy are primarily fever, anemia and blood clotting problems (Michalsen, 2009). Treatment using leeches is relatively cheap and safe, and it often turns out to be a final solution when conventional treatments fail.

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Limitations of serological screening of *Toxoplasma gondii* infection

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Congenital toxoplasmosis results from a primary infection with *Toxoplasma gondii* during pregnancy. Serological screening during pregnancy can lead to an early diagnosis of the infection and help to implement treatment, which slows down the disease progression in infected children. Tests for detecting the IgG antibodies are used for screening purposes. Conversion from a negative to a positive titer or a rise from low to a significantly higher titer in serial serum specimens is observed in the acute phase of the infection. The study was designed to assess tests for the demonstration of IgG antibodies used in serological monitoring of acute *Toxoplasma gondii* infection.

Four serological tests for detecting *T. gondii* – specific IgG antibodies were used: ARCHITECT Toxo IgG (Abbott), VIDAS Toxo IgG II (bioMérieux), COBAS Toxo IgG (Roche) and immunofluorescence indirect IF (in-house NIPH-NIH). The results of the tests were expressed in international units (IU). Statistical analysis was performed using a paired samples t-test.

Discrepancies of results obtained from a sample with low IgG titer were found: ARCHITECT (titer IgG 2.4 IU/ml) and VIDAS (4 IU/ml) tests results were ambiguous, while the results of COBAS (60.2 IU/ml) and IF (12 IU/ml) were positive. The difference between median IgG titer value measured by COBAS (562.5 IU/ml) and ARCHITECT (41.6 IU/ml) and titer values from other tests is statistically significant: COBAS – IF ($p=0.000$), COBAS – ARCHITECT ($p=0.000$), COBAS – VIDAS ($p=0.000$), ARCHITECT – IF ($p=0.056$), ARCHITECT – VIDAS ($p=0.000$). There were no statistically significant differences in median IgG values in serum when samples were tested using VIDAS (137.6 IU/ml) and IF (141.2 IU/ml) ($p=0.910$) assays.

Changing tests for the detection of *T. gondii* – specific IgG antibodies during serological screening in pregnant women can disrupt the diagnostic process of acute *Toxoplasma* infection.

A preliminary study on the occurrence of enterobiasis among children from Olsztyn (north-eastern Poland)

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Enterobiasis is one of the most frequent human parasitic diseases caused by the intestinal parasite *Enterobius vermicularis* (the pinworm). Enterobiasis may be asymptomatic or cause perianal pruritus, insomnia, restlessness and irritability. Enterobiasis predominantly occurs in school-aged children (5–14 years). According to a nationwide study conducted during the school year 2002/2003, the highest percentage of infected children was in the Warmia and Mazury province, where enterobiasis was diagnosed in 30.7% of 7-year-old children (Bitkowska et al. 2004). In Olsztyn, recent studies on the occurrence of enterobiasis in children aged between 5-7 years were performed during the school year 1996/1997, and showed that 12% of students had an infestation of *E. vermicularis* (Żółtowska & Danieluk 1999).

The aim of this study was to evaluate the prevalence of enterobiasis among children from the Olsztyn area. A group of 112 children (3-14 years) from one nursery (50.9%) and one primary school (49.1%) in Olsztyn were examined using the adhesive cellophane tape method. Perianal swabs were collected three times from each child by the parents in accordance with clear instructions. Infestation with the parasite was confirmed by microscopic detection of *E. vermicularis* eggs on slides. Data on the socio-economic status of children as well as symptoms suggestive of enterobiasis were collected using a self-designed diagnostic questionnaire.

The overall prevalence of *E. vermicularis* was low. *Enterobius* eggs were detected only in 6 children (3 girls and 3 boys, 5.3%) out of 112 examined. Four of the infected children were elementary school pupils aged 7 (n=2), 8 (n=1) and 14 (n=1) years. The remaining two children were preschoolers aged 5 and 7 years. In two of the infected children, enterobiasis was asymptomatic. The parents of the other four children observed anal itching, abdominal pain, pale skin and lack of appetite in their children. These symptoms were also reported in children with a negative cellophane test result: anal itching (13.2%), abdominal pain (16%), pale skin (18.9%) and lack of appetite (11.3%).

The prevalence of *Demodex* spp. infestation in students from the Faculty of Medical Sciences at the University of Warmia and Mazury in Olsztyn – preliminary study

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Demodex mites are common permanent ectoparasites of the mammalian pilosebaceous unit. In human skin, two species are present, *D. folliculorum* and *D. brevis*, and they live in slightly different niches: the former in hair follicles and the latter in sebaceous glands. However, they often occur simultaneously in the same host. Usually considered to be harmless saprophytes, they can multiply and possibly play a role in the formation of skin lesions under favorable conditions.

A group of 95 students from the University of Warmia and Mazury in Olsztyn was examined for the presence of *Demodex* spp. in eyelid and facial skin hair follicles. Two eyelashes from each eye were epilated from 91 study participants. Furthermore, the contents of 2 to 3 sebaceous glands were extracted from the forehead, nose and chin of 82 study participants. All material was placed in Hoyer's liquid, then the slides were examined microscopically. The presence of eggs, adults or nymphal and larval forms was considered to be a positive result. The appearance of symptoms, existing dermatoses and the type of skin were taken into account.

The study showed that 2% of people were found to be carrying *D. folliculorum* in the hair follicles of the eyelashes, while 23% of the students' skin specimens were positive for *Demodex*: 9.7% for *D. folliculorum*, 7.3% for *D. brevis* and 6% were carriers of both *Demodex* species. In 9 cases (47%) a combination skin type was reported, in 7 cases (37%) – normal skin. Only 2 persons (11%) noted oily skin and 1 person (5%) had dry skin. All students having *D. folliculorum* in the eyelid hair follicles were asymptomatic, as well as 26% of the skin carriers. Others reported the following symptoms on their facial skin: pustules, purulent pimples, red spots and pruritus.

The prevalence of *Demodex folliculorum* and *Demodex brevis* among the inhabitants of north-western Poland

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The aim of this study was to evaluate the prevalence of *Demodex folliculorum* and *D. brevis* among the inhabitants of north-western Poland. In addition, associations between the presence of *Demodex* spp. and host factors such as age, sex and demodicosis symptoms, were analyzed.

Ninety-three patients of the parasitological laboratory with symptoms of blepharitis, ninety-five patients of the Clinic of Haematology, Pomeranian Medical University, and a control group of 111 healthy individuals were included in this investigation.

Demodex spp. was found in 53.8% of patients of the parasitological laboratory, in 20% of patients with blood disorders and in 11.6% of controls. The difference between the control group and the parasitological laboratory patients was statistically significant. The overall prevalence was 27.3%. In all groups, a correlation was confirmed between the presence of *Demodex* spp. and the age of the patients. The highest percentage of *Demodex* spp. infection in all groups studied was reported in people over 71 years of age. The main symptoms of demodicosis included tearing, itching and burning eyes, redness of the eye and the area around the eyes, and burning and itching of the eyelids. A correlation between the presence of cylindrical dandruff in eyelashes and the occurrence of *Demodex* spp. was found in all groups.

Consequently, it was concluded that the incidence of *Demodex* spp. was high in patients with blepharitis compared with the control group and patients with blood disorders. The relationship between the prevalence of *Demodex* spp. and age of host and occurrence of cylindrical dandruff was confirmed.

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Dirofilariosis – 8 years from the first finding in the territory of Slovakia

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The first systematic epidemiological research of canine dirofilariosis in Slovakia was initiated in 2007, two years after the first detection of the parasite in dogs from south-western part of the country. The primary aim of the research was to determine whether climatic conditions in Slovak territory are favourable for development and spread of autochthonous dirofilariosis.

Between 2007 and 2012 more than 3000 dogs were examined using Knott test and PCR for *Dirofilaria* spp. presence. Mean prevalence rate of about 12.00% was found; the majority of infected dogs came from southern regions of the country. *Dirofilaria repens* was confirmed as etiological agent of infection in all positive samples, in 8 dogs mixed infection with *D. repens* and *D. immitis* (heartworm) was revealed.

The objective of the research in the second phase was to ascertain the presence of wildlife source of dirofilariosis in ecological conditions of Slovakia. DNA isolated from spleens of 183 red foxes was remitted to PCR analysis. The results showed that 105 (57.4%) out of the examined foxes were infected with *D. repens*. Most test positive foxes (87 out of 110) were hunted in the region of south-eastern Slovakia. BLAST analysis of the obtained sequences showed 100% similarity between all isolates from foxes and the sequences of *D. repens* isolated from humans, cats and dogs deposited in GenBank. Molecular analyses confirmed also the first finding of *D. repens* in beech marten (*Martes foina*) from the Tatra National Park, northern Slovakia.

The results of epidemiological survey revealed that environmental circumstances in the whole territory of Slovakia are favourable for circulation of *Dirofilaria* parasites and their vectors, and the infection risk exists for both animals and humans. The first human case of subcutaneous dirofilariosis in Slovakia was diagnosed in 2008 and since then four other cases have been reported.

The work was realized within the frame of the project funded by the Science Grant Agency VEGA 2/0011/12 and project APVV SK-PL-0098-12.

Occurrence of *Trichinella* spp. in wild carnivores in Poland: up-to-date

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Trichinellosis is a zoonosis caused by parasitic nematodes of genus *Trichinella*. Members of the genus are able to infect a broad spectrum of mammalian hosts, which makes them one of the world's most widely distributed groups of nematode infection. A total of 12 genotypes have been identified in the genus *Trichinella*. The majority of *Trichinella* spp. cause disease in humans. Four species of *Trichinella*: *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis* are represented in Poland.

The aim of the study is a complex and up to date research on the parasites spreading in wild carnivores in Poland. The study was performed on 1138 wildlife carnivores from different regions of Poland collected in 2011–2013. Muscle samples were taken from diaphragm pillars, the tongue and the upper part of the front legs. Each sample was examined individually using artificial pepsin-HCl digestion method by Cabaj and Przyjałkowski (1987) and Gamble *et al.*, (2000). Muscle larvae were identified as *Trichinella* based on gross morphology. Genomic DNA was extracted from single larvae using Nucleospin Tissue DNA extraction kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. *Trichinella* larvae were identified at species level by multiplex polymerase chain reaction (multiplex PCR) according to Zarlenga *et al.* (1999). *T. britovi* was identified as a dominant species. The overall prevalence in the examined samples was found to be 5.7%. Muscle larvae were found in 10.7% of 465 red foxes (*Vulpes vulpes*), in 7.1% of 14 Eurasian badgers (*Meles meles*), in 5% of 20 pine martens (*Martes martes*), in 2.27% of 509 wild boars (*Sus scrofa*) and in 3.3% of 35 raccoon dogs (*Nyctereutes procyonoides*). No *Trichinella* larvae were detected in 60 raccoon (*Procyon lotor*), 3 Eurasian otters (*Lutra lutra*) and 8 muskrats (*Ondatra zibethicus*), 17 American minks (*Neovison vison*), 4 hedgehogs (*Erinaceus* spp.), 2 forest rats (*Avicola terrestris*), 1 polecat (*Ondatra zibethius*).

Our results reveal that *T. britovi* can invade a broad spectrum of sylvatic carnivores in Poland. The infection can be transmitted to wild boars and, consequently, it can easily reach the humans. This is especially important in terms of very dynamic changes in populations of such animals as: the foxes, raccoon dogs or the American mink.

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Cases of infection of *Sarcoptes scabiei* in wild carnivorous

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Scabies is a parasitic disease caused by burrowing mites (*Sarcoptes scabiei*). Material of the study constituted two red foxes (*Vulpes vulpes*) and one pine marten (*Martes martes*). The foxes was hunted in the hunting season 2012/2013. The pine marten was caught in a basement where the owner fed homeless cats.

In the first fox erythema with papule formation and alopecia, especially on thighs, shanks and forepaws, were observed. The skin was inflamed and acanthosis was slightly increased. In the second fox acanthosis and alopecia on the abdomen and tail were found. Despite clear signs of scabies, the foxes were in good body condition with well developed subcutaneous adipose tissue.

In the pine marten extensive pathogenic changes with alopecia, acanthosis and crust formation, especially on head, neck, back and tail, were observed. The hair on the trunk was matted and discolored. Eyelids were bonded by inflammatory secretion. The animal was emaciated and in poor condition.

Eggs and adult forms of sarcoptic mange mites (*Sarcoptes scabiei*) were found in skin scrapings and skin biopsies taken from the foxes and marten.

Keratitis incidences difficult to diagnose and treat suspected of amoebic aetiology in contact lens using patients

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We assess keratitis cases suspected of amoebic aetiology in contact lens wearers and evaluate the usefulness of *in vitro* techniques for the prognosis of treatment efficacy.

Six female contact lens users, 26–41 years old, some previously treated unsuccessfully, admitted to our hospital in the period 2009–2012 were retrospectively analyzed. All patients complained of great pain and photophobia. An inflammation and a large, deep corneal ulcer under the epithelial defect were detected. Slit-lamp, *in vivo* confocal microscopy, microbiological and parasitological examinations were performed. An initial diagnosis was made by *in vivo* confocal microscopy; the final diagnosis was confirmed by corneal scrapings examination in the light microscope and further *in vitro* cultivation.

Acanthamoeba keratitis was identified in 4 patients. Mixed bacterial-fungal-amoebic infections occurred in 2 of these patients. Amoebic cysts were detected by *in vivo* confocal microscopy in 2 patients, while *Acanthamoeba* trophozoites developed in the corneal scraping cultures of 4 patients. Prolonged pharmacotherapy was undertaken in 4 cases; surgical procedures were necessary in 3 of them (keratoplasty, corneal grafting).

The mixed aetiology of some keratitis cases – and the late recognition of amoebic infections – were the factors influencing treatment difficulties. The variability in symptom intensity, as well as the differences in resistance to pharmacotherapy and surgical management efficacy which appears in incidences of *Acanthamoeba* keratitis correlate with the surviving time of cultivated amoebae. The *in vitro* monitoring of the dynamics of *Acanthamoeba* strains isolated from the eyes may be useful tool for proper diagnosis and prognosis of treatment efficacy, particularly in contact lens wearers.

Usefulness of intra-operative material assessment for verification of diagnosed or suspected cases of human echinococcoses

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In this study, cases of liver lesions suspected of echinococcal aetiology requiring surgical treatment were assessed retrospectively. The data of five patients admitted to our surgical departments in 2011 with abdominal discomfort and severe liver dysfunction were analyzed: 37 to 68-year old, four females and one male. The patients underwent angio-computerized tomography (angio-CT), abdominal ultrasonography (US) and serological tests. Some of them had been conservatively treated for several years with albendazole before hepatectomy due to *Echinococcus* infection. Intra-operative liver samples were examined parasitologically and histopathologically to confirm or verify diagnosis.

In the patients, pre-operative angio-CT and US revealed hepatic lesions, abscess, tumor-like masses or cysts. In the 37-year old woman, the presence of partially calcified fragments of *E. granulosus* cyst wall (CE 5 type by WHO classification) was detected; rostellar hooks and protoscolices in the liver samples were revealed microscopically. The cystic echinococcosis was also confirmed in the 64-year old woman. In the 68-year old man with tumorous liver lesions, fragments of larval laminated layer typical of alveococcosis were histopathologically found. A liver abscess of bacterial aetiology was identified in the 60-year old female patient. In the 68-year old woman, with a tumor-like mass detected by angio-CT and US, which had been treated for *E. granulosus* without effect for two years, examinations of liver samples verified previous diagnosis: changes in liver were revealed as early cystadenocarcinoma muciparum.

Unspecific clinical symptoms, uncertain findings of imaging techniques and late proper diagnosis are factors which cause difficulties in the successful treatment of echinococcosis. Thus, intra-operative material assessment is helpful particularly for confirmation/verification of suspect cases difficult to diagnose and treat.

The usefulness of histopathological examinations of the lesions diagnosis in animals caused by the mites (Acari) and larval forms of insects (Insecta)

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Histopathological examination is important in the diagnosis of human and animal diseases of varying etiology, especially in the diagnosis of cancer. The demonstration of histopathological changes during certain parasitic invasions is particularly important for differential diagnosis and often confirms the presence of parasitic diseases. Skin biopsies from dogs, slices of mucosa from horse stomachs and slices of mucosa of the frontal sinus of sheep were taken for histopathological examinations. Materials were fixed in 8% formalin. Histopathological formulations were stained with haematoxylin and eosin (H-E).

In dogs, burrowing mites (*Sarcoptes scabiei* v. *canis*) parasitize the deep layers of the epidermis (*stratum granulosum*, *s. spinosum*). Changes are located on the head, rump and at the base of the tail. At the beginning of the invasion, lesions are found in the form of local, limited exfoliation of the skin, which next creates scaly raids. During the course of the disease, the skin is reddened and thickened, and papules were formed in the corneous epidermis. *Demodex canis* occupies the hair follicles and sebaceous glands and causes the creation of small nodules with thinned hair, and excessive desquamation of skin. Initially, the changes appear on the head, then small lesions form a larger, clearly demarcated, red scalp, extending to the neck, forelegs and the rest of the body. The disease is chronic.

The presence of *Oestrus ovis* larvae causes inflammation of the mucous membrane of the nasal cavity and sinus. Occasionally the larvae may penetrate the bones of the skull and enter the cerebral cavity – clinical signs are similar to those caused by *Taenia multiceps* called false gid. Changes in the nasal tissues include the catarrh infiltration of inflammatory cells and squamous metaplasia.

At the point of attachment of the L2 and L3 larval stages of *Gasterophilus intestinalis*, in the glandular and non-glandular stomachs of horses, are wastages on both the corneous layer as well as the squamous mucosa and its focal hyperkeratosis.

Colonies of bacteria were observed in the necrotic foci, as was infiltration of eosinophils and neutrophils. Diffuse infiltrates of inflammatory cells were visible at the bottom of the wastage in the proper epidermal layer of the mucous membrane.

Cutaneous dirofilariosis in a dog

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Dirofilariosis is a disease of dogs and cats found in areas of the Mediterranean, Asia, America, Africa and Australia. The first cases of canine dirofilariosis were described in Slovakia in 2005, in the Czech Republic in 2006 and in the Netherlands, Germany and Austria in 2009. There are also descriptions of cases of *D. immitis*, and indigenous cases of *D. repens* in Poland. In 1994, the author detected the microfilariae in the blood and cerebrospinal fluid in a dog (crossbreed) with neurological symptoms from Trzebnica.

The adult nematodes of *D. immitis* are localised in the right chamber of the heart and the pulmonary artery, and *D. repens* are present in subcutaneous and intermuscular connective tissue.

The larvae (microfilariae) are transmitted by intermediate hosts – mosquitoes of the genera *Aedes*, *Culex*, *Anopheles* and *Myzorrhynchus*. In the gut of an insect, the microfilariae penetrate the Malpighi tubules, reach the invasive stage (L3) within 11–14 days and then return to the insect body cavity to the lower lip. The larvae penetrate the skin of the definitive host, moult twice and reach the adult stage about 6–9 months after sexual maturity. Mature worms live for 2–4 years. The adult *D. repens* can cause multifocal, nodular inflammatory changes on the skin.

A case report. In December of 2012, multifocal, nodular inflammatory changes on the skin around the shoulder were diagnosed in a German Shepherd Dog originating from Konin. The biopsy of the skin revealed the presence of microfilariae. Microfilariae were detected in a blood sample collected on EDTA and tested according to Knott, Kingston and Morton methods. A SNAP 4DX (IDEXX) test for the presence of circulating antigens of *D. immitis* was negative. Molecular analyses with the use of *D. repens*-specific primers (5'-CCGGTAGACCATGGCATTAT-3' and 5'-CGGTCTTGGACGTTTGGTTA-3') confirmed *D. repens* infection.

Histopathological diagnosis of parasitic diseases

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The histopathological examination plays an important role in the diagnosis of human and animal diseases of different etiologies. The aim of the study was to determine the role of histopathology in the diagnosis of animal parasites and demonstrate pathological changes in the course of parasite invasions caused by different taxonomic groups of parasites: Protozoa, Trematoda and Cestoda. The tissue samples were collected from various animal species suspected of having a parasitic invasion. The obtained material was fixed in 8% formalin. Histological tissue samples were stained with haematoxylin and eosin (H-E). The histopathological analysis of the large intestines and livers of infected turkeys revealed numerous oval or circular shaped *Histomonas melagridis* protozoan parasites with granular endoplasm. The histopathological examination of the large intestine and liver of reptiles revealed the *Entamoeba invadens* parasite. These protozoan parasites have an oval shape with a well-stained nucleus and karyosome. In pigs infected with *Balantidium coli*, the intestinal wall cross-sections revealed numerous round or oval protozoan parasites with bean-shaped macronucleus. The protozoan parasites surrounded the inflammatory infiltrate, which was composed of lymphocytes, histiocytes and eosinophils. In rabbits suffering from coccidiosis, the schizogony and gametogony damaged the biliary epithelium and produced clinical symptoms. In some species of rabbit coccidia schizogony and gamogony take place in the small or large intestine producing inflammatory changes detectable histologically. Thin gamonts of *Eimeria necatrix*, *E. acervulina*, *E. precox*, *E. mivati*, *E. maxima*, *E. brunetti* and *E. tenella* were visible within the epithelial cells of the small and large intestinal mucosa. Intermediate host skeletal muscle cross-sections revealed many zoites (*Sarcocystis* sp.) within sarkocysts (Miescher cysts). The juvenile *Fasciola hepatica* fluke injures the hepatic parenchyma instigating hemorrhage when moving to the bile ducts. The *Schistosoma haematobium* fluke eggs utilize their spine to penetrate the wall of the urinary bladder and enter the external environment with the host's urine. The *Schistosoma mansoni* fluke eggs break through the wall of the colon into the gastrointestinal tract and in doing so damage the lamina and mucosa of the large intestine. The masses of eggs become surrounded by inflamed areas infiltrated by leucocytes, particularly eosinophils. The larval form of *Taenia pisiformis* burrows tunnels through the hepatic parenchyma of rabbits, damaging their liver parenchyma and leading to the formation of a peripheral inflammatory infiltrate composed of eosinophils and giant cells. In chronic cases, the damaged liver parenchyma produces fibrotic scars. The larvae of *E. multilocularis alveolaris* are often localized in the liver, lungs or brain. Inflammatory infiltration with numerous eosinophils, necrosis and calcification grows around the larval forms. Histological detection of the hooks of *Echinococcus* larvae determines the diagnosis. The histopathological examination is useful for a number of reasons: a/ for the detection of parasites b/ to reveal the area of tissue damage caused by migrating larval forms and mature parasites, c/for the implementation of appropriate treatment, d/ to explain why certain treatment is not effective during a parasite invasion (due to parasites damaging parenchymal organs, causing permanent organ dysfunction and the formation of fibrotic scars).

The occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in treated effluent from sewage treatment plant from eastern Poland

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Cryptosporidium spp. and *Giardia lamblia* are parasitic protozoa causing diarrheal disease in humans. Sewage is an important source of these protozoans. Depending on the type of technological processes applied in sewage treatment plants, the removal of these parasites from the final products of the technological process, including water released into the environment, can be different.

The aim of this study was estimate the prevalence of *Cryptosporidium* oocysts and *Giardia* cysts in wastewater (effluent) from selected sewage treatment plants located in eastern Poland, with regard to surface water contamination and as a potential threat to human health. Samples of treated water (effluent), each of 10 L volume, were collected from 13 municipal wastewater treatment plants (WTP) located in eastern Poland. After centrifugation of samples, parasite isolation was performed from obtained sediments using immunomagnetic separation (IMS). Part of the IMS product was examined microscopically with the use of direct immunofluorescence (DFA), and DNA was isolated from the remainder. For selected samples, an evaluation of viability for isolated (oo)cysts was also carried out using the commercial kit Live/Dead BacLight (Invitrogen). The molecular identification of *Giardia* was conducted based on reaction nested PCR, where the fragment of the β -giardin gene was amplified (according to Caccio and al., 2002). For detection of *Cryptosporidium*, the fragment of the 18 SSU rRNA gene was amplified in nested PCR (according to Xiao and al., 2001). DNA samples were also examined with Real Time PCR, in which fragments of *Giardia* genes and *Cryptosporidium*: β -giardina (74 bp) and COWP (151 bp) were amplified (according to. Guy and al., 2003).

In DFA, *Cryptosporidium* spp. oocysts were detected in samples from 8 WTPs (61.5%) and *Giardia* spp. cysts in samples from 11 WTPs (84.6%). Parasites of both genera were detected in 7 samples. In the examination of the samples coming from 4 selected sewage treatment plants, living *Cryptosporidium* and *Giardia* were detected in 4 and 3 samples, respectively. In 4 of 13 samples (30.8 %) tested in nested PCR, *Giardia* was detected, however *Cryptosporidium* was found in none of them. In Real Time PCR, the presence of *Giardia* and *Cryptosporidium* DNA were detected in 5 (38.5%) and 1 (7.7%) of the samples, respectively. The detection of *Cryptosporidium* and *Giardia* in treated effluent originating from the majority of studied WTPs, indicates a potential threat to public health and the necessity of implementing additional cleaning procedures.

Infestation of a mandibular gland by *Trichomonas tenax* in dog. A case report.

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Trichomonadea, commonly called trichomonads, comprise a class of amitochondriate protists belonging to the phylum Parabasalia. They are morphologically characterized by multiple anterior flagella and a single recurrent flagellum that functions as an undulating membrane. Among these microorganisms the most frequent are commensal protozoan in the large intestine. Several are considered to be intestinal or urogenital parasites of humans and animals including carnivores such as dogs. Only a few species were typically found in the oral cavity of animals. PCR based molecular analysis of the isolates obtained from a dog, mostly in Europe, confirmed the presence of *Trichomonas tenax*, *Trichomonas* spp., *Tetratrichomonas canistomae* and *Tritrichomonas foetus* in the oral cavity. The incidence of oral trichomoniasis in dog is approximately 15–25%, although the prevalence varies in different populations depending on age, clinical condition of the oral cavity and sex. There is also a significant correlation between the occurrence of this invasion and periodontal diseases. This is a case report of infestation of the mandibular gland by *Trichomonas tenax* in a 13-year-old dog with advanced stages of periodontal disease. The dog with a history of a painless left mandibular gland nodule that had gradually increased in size was admitted to the veterinary clinic at the University of Life Sciences in Lublin. According to the owner, the dog suffered from chronic halitosis which caused periodontitis. Clinical, radiological and biological investigations were fruitless. In the collected swab of the oral cavity multiple mobile trophozoites of trichomonads were found. In post-mortem investigation, the aspiration from pathological changes of the left mandibular gland also revealed numerous trichomonads. The obtained materials were cultured on Pawłow and Simič medium. Positive cultures were used for taxonomical classification based on scanning electron microscopy study and molecular diagnostics (PCR method). The ITS1-5,8S-ITS2 region analysis confirmed the presence of *T.tenax*, which led to the conclusion that the mandibular gland was infested by *Trichomonas tenax*. According to our knowledge this is the first report of trichomonads isolation from salivary glands in dog. This case sheds new light on the pathogenic properties of trichomonads localized in the oral cavity of carnivores.

Molecular detection of *Dirofilaria repens* and *Wolbachia* spp. in cats from Warsaw – preliminary studies

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Dirofilariosis due to the nematode of *Dirofilaria* genus is globally widespread and is a leading cause of morbidity and mortality in dogs and cats in endemic areas. The prevalence in cats is consistent with the prevalence in dogs in a given area, but usually lower in percentage terms (5 to 20%). It is believed that cats are more resistant to *Dirofilaria* infections, which is possibly linked with the host-feeding preferences of the mosquito. Filarial nematodes harbor *Wolbachia* endosymbionts that play an important role in the pathogenesis of filarial infections in mammals. *Wolbachia* is an intracellular Gram-negative bacterium belonging to the order Rickettsiales and found in 20 to 80% of arthropod species and in the nematodes of Onchocercidae family. The nematode harbors thousands of bacteria, and the bacteria are released when the parasite is killed by specific therapy or dies spontaneously in the host. As a result, a PCR targeting *Wolbachia* DNA would be more suitable than a PCR targeting directly *Dirofilaria* DNA.

In Poland, the autochthonous dirofilariosis was found in humans for the first time in 2012. The cases of canine dirofilariosis have been observed since 2007 in Warsaw and in its surroundings (the Mazovia province). The prevalence of *D. repens* in dogs ranges from 21 to 60%. Up to date, nothing is known about *Dirofilaria* infections in cats. The aim of our preliminary study was to evaluate and compare the detection of filarial and bacterial DNA in feline blood as alternative assays for diagnosing feline filaria-associated syndromes in cats.

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