STRESZCZENIA REFERATÓW I KOMUNIKATY

BIOLOGICAL POLLUTION OF ENVIRONMENT AS A THREAT TO PUBLIC HEALTH

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The growing contamination of the environment creates a lot of public health threats. In recent years infections due to intestinal protozoan parasites such as *Giardia*, *Cyclospora*, *Cryptosporidium* as well as microsporidia have become a serious threat to human and animal health. The dispersive stages of the parasites excreted with the faeces of infected hosts are one of the components of biological pollution of the environment. The main causes of the biological contamination of the environment, particularly of water ecosystems, are sludges from combined sewerage systems, agricultural runoff, as well as faeces of livestock. Also, faeces of wild animals may cause contamination of surface waters. Besides other ways of infection by intestinal protozoan parasites the attention of both scientists and the media has been drawn to many waterborne outbreaks of giardiosis, cryptosporidiosis, cycloporosis, and intestinal microsporidiosis. Water, which is the most valuable natural resource is also the main agent causing waterborne diseases. It is estimated that waterborne diseases kill 12 million people per year, most of them in developing countries. In both developed and developing regions waterborne outbreaks due to intestinal protozoan parasites have also been recorded because contemporary water treatment methods are insufficient to eliminate intestinal pathogens. Moreover, even the sophisticated methods used for the detection of oocysts and cysts in water samples are insensitive. Thus, at present it cannot be guaranteed that drinking and recreational water is devoid of dispersive stages of intestinal protozoan parasites. Therefore, multiple barriers are needed to protect our water supplies against biological contamination.

ACANTHAMOEBA SPP. AS VECTORS OF PATHOGENIC BACTERIA

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Free-living amoebae of *Acanthamoeba* genus are cosmopolitan organisms. Their habitat is humid soil and all water reservoirs, including fresh-, see-, ice- and hot water. They mainly feed on bacteria. Pathogenic properties of amoebae as well as the mechanisms of infections caused by them have not yet been fully described. Amoebae cause granulomatous amoebic encephalitis, pneumonia, keratitis, and infections of other tissues. Only few isolates are strongly and permanently pathogenic and infectious to humans. Some isolates lose their pathogenic properties even after one passage. It is assumed that such short-lasting pathogenic properties of amoebas may be caused by microorganisms living inside the amoebas. It is a common knowledge that free-living amoebae may be naturally infected with pathogenic bacteria, which can live and proliferate in their cells.

The aim of our study was the evaluation of the pathogenic properties of amoebae of *Acanthamoeba* genus isolated from the Malta Lake in Poznań and from tap water collected at surgical wards of Poznań clinical hospitals. In addition, the study attempted to find a connection between the properties of amoebae and the presence of pathogenic bacteria in water or inside the amoebae.

For the purpose of amoeba isolation and proliferation NNE agar medium was used covered with dead bacteria. Pathogenicity was determined following intranasal inoculation of mice of BALB/c strain with amoeba trophozoites. Isolates were identified based on the biochemical properties and morphological characteristics of the trophozoites and cysts. The presence of bacteria in water, in amoebae and in tissues of dead mice was confirmed using bacteriological and immunological methods. Changes of the tissues were confirmed using histopathological methods.

It was found that a majority of the isolated amoebae were those of *A. castellanii* and *A. rhysodes*. Most of the isolates appeared pathogenic to mice. Parasites were isolated from brains and lungs. Bacteriological assays revealed that approximately 50% of amoeba isolates contained in their cells both pathogenic and non-pathogenic bacteria (including, among others: *Proteus mirabilis*, *Proteus vulgaris*, *Clostridium perfrigenus*, *Escherichia coli*, and even *Salmonella* sp. and *Legionella pneumophila*).

The presence of bacteria inside free-living amoebae poses a real challenge to bodies responsible for testing and inspecting the quality of surface waters, swimming pools, and drinking water intakes. It is highly possible that the occurrence of some diseases in humans may be related to the presence of bacteria in amoebae (e.g. infections contracted at hospitals).

PATHOGENIC PROPERTIES OF FREE-LIVING AMOEBAE ISOLATED FROM ARTIFICIAL WATER BODIES IN THE CITY OF SZCZECIN

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The aim of the present study was to assess the pathogenic properties of the amoebae isolated from artificial bodies of water, situated in Szczecin. This project covered 10 indoor swimming pools and 3 outdoor, seasonally open, swimming complexes.

A total of 30 strains of amoebae of the genus *Acanthamoeba* were isolated from 46 water samples. Fifteen of the above-mentioned strains demonstrated thermophilic properties. Eight of them represented indoor swimming pools while 7 – seasonal swimming complexes. Pathogenic properties and the virulence level of the thermophilic strains were tested on mice of the Swiss strain. Two-week-old mice were infected through nasal inoculation with 0.05 ml of the trophozoite suspension (some 25 thousand) from an in-vitro culture.

The results of the present study demonstrated pathogenic properties of 3 strains of *Acanthamoeba* from outdoor swimming complexes: AM-16, AD-17, GO-3.

After 7-30 days post infection, the presence of trophozoites of the pathogenic strains was recorded in the brain, liver, kidney, and the spleen of the infected animals. The infected organs were also studied histopathologically.

The presence of thermophilic amoebae, pathogenic for mice in the recreational bodies of water in Szczecin indicates a possible risk of infection for people using those swimming complexes.

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HELMINTHOLOGICAL STUDIES OF SEWAGE SLUDGE DESIGNED FOR AGRICULTURAL USE

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In recent years, ecological awareness of the society has increased. There is a tendency to repair what has been destroyed by human activity. All elements of the environment have been devastated, including soil. The latest data show that as the result of human economic expansion more than a half of soils in the world have been affected by degradation. The problem of soil contamination becomes especially important while talking about ecological agriculture. Ecological agriculture is accepted by society as it is for a consumer a warranty of health food. Therefore, there is a demand for safe and proper application in agriculture of secondary raw materials (sewage sludge) in the form of fertilizers.

Sewage sludge used for the production of fertilizers must fulfil precisely defined sanitary requirements, such as:

(1) lack of live eggs of intestinal parasites; (2) lack of pathogenic microbes, mainly *Salmonell*a bacteria, (3) not exceed allowable concentrations of heavy metals.

Sewage sludge is usually subject to the composting process, which, if carried out in a proper way eliminates the presence of live eggs of intestinal parasites and other biological markers.

Despite economic benefits, the application of sewage sludge in agriculture leads to biological contamination of soils, and sometimes vegetables cultivated in these soils. The parasitological markers of this pollution are eggs of *Ascaris* spp., *Trichuris* spp., and *Toxocara* spp.

During the period 2001-2002, a total of 55 samples of fermented and dehydrated sewage sludge designed for agricultural use were examined. The sludge came from sewage treatment plants in various regions of Poland. Eggs of *Ascaris* spp. and *Trichuris* spp. were detected by flotation method according to Wasilkowska, whereas eggs of *Toxocara* spp. – by flotation method by Quinn et al. Eggs of *Toxocara* spp. were most frequently found – in 14.5% of the samples examined, followed by eggs of *Ascaris* spp. – in 9.1%, and eggs of *Trichuris* spp. – in 7.2% of the samples.

The results of the studies indicate that sewage sludge may be the source of biological contamination of soil.

DOGS, HELMINTHS AND URBANIZATION

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Progress and development of the modern civilization is closely joined with urbanization. In developing countries the unplanned, uncontrolled and constant migration of people from the rural areas to the cities results in an increase of the sanitation and health problems. In overcrowded agglomerations, among poor people, many infected and parasitic diseases caused by pathogenic organisms being found in human faeces are spread. In contrast, in cities of developed countries the main culprit of biological soil contamination are pets and especially dogs whose number is big and still growing up quickly. Dogs always were recognised as friends of people and played an important role in societies through-

out the world. They are useful companions in many households, contributing to the physical, social and emotional development of children and the well-being of their owners. Although pets offer significant benefits to our society, they also provide some health hazards such as: bites, allergy, and a diverse range of zoonotic infections.

The most prevalent zoonosis transmitted from dogs to humans are helmithoses – toxocarosis caused by *Toxocara canis* and ancylostomosis caused by *Ancylostoma caninum*. Both species are common cosmopolitan nematodes but our special attention attracts *T. canis* because its prevalence among pups reaches 100%. Moreover, toxocarosis among people relatively often manifests the serious clinical symptoms – visceral (VLM) or ocular (OLM) *larva migrans*. In Poland about 8.8 million dogs live in human households. In cities, a huge population of dogs becomes increasingly troublesome, mainly because of their faeces which contaminate soil of public places. This is not only unsightly but also dangerous for epidemiological reasons. Soil contaminated with *Toxocara* spp. eggs is the simplest indicator of the risk of human toxocarosis. The eggs do not penetrate soil profile easy; the majority of them stay on the superficial layer of the ground and can survive there as many as 6 years. Therefore they persist in soil and may be treated as an indicator of the sanitation level of the environment. The most contaminated areas with *Toxocara* spp. eggs are city backyards where children often play. In Poznań, the eggs are being detected in high numbers within the past 10 years regardless of the year season (16-50% positive soil samples). It must be emphasised that a correlation of complex nature between soil contamination and the prevalence of toxocarosis among people was observed. Among other factors influencing the infection can be the species of parasite. Most probably the role of *T. cati* in human toxocarosis is underestimate but this problem needs further studies.

There is no doubt that in Poland for aesthetic and epidemiological reasons the activities for improving the sanitation of cities should be undertaken but the task is complex and not easy. It requires to coupe with follow problems: the huge number of dogs and cats (especially homeless), their systematic disinfection, prevention of public contamination with pet faeces, and to develop of educational programs aimed at increasing public awareness of potential zoonotic threats. The best effect can be expected if all these steps are taken jointly.

DEPENDENCE OF CLINICAL CASES OF TOXOCAROSIS IN CHILDREN UPON THE CONTAMINATION OF ENVIRONMENT WITH *TOXOCARA* SPP. EGGS IN MAZOWIECKIE VOIVODSHIP

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Human toxocarosis is caused by an infection with dog- or cat round worm *Toxocara* spp. larvae. After ingestion of eggs by the patient, the infecting larvae begin a somatic migration through the inner organs. Larvae remain most frequently located in the liver and the lungs but can also cause serious ocular damage by migrating into the retina. Children are the most exposed for infection, because of their close contact with dogs and cats, specific behaviour (geophagy) and lower level of resistance when compare to adults. According to larvae locality toxocarosis can appear as: visceral form (*visceral larva migrans)*, ocular form (*ocular larva migrans*) or covert toxocarosis. The clinical manifestation of toxocarosis depends on the number of larvae ingested and frequency of infection, distribution of larvae in the body, and intensity of the host's immunological response. Several hundred cases of toxocarosis mainly in children are recognised in Poland each year.

In the the frames of the research project financed by the State Committee for Scientific Research („Dependence of clinical cases of toxocarosis in children upon the prevalence of *Toxocara* spp. in dogs and cats and contamination of environment with *Toxocara* eggs") an environmental study has been carried out in cooperation between the Institute of Parasitology of the Polish Academy of Sciences, Clinic of Ophtalmology, Clinic of Infectious Disease, and the Clinic of Zoonoses and Tropical Diseases, the Medical University in Warsaw.

Home environments of children, hospitalised/treated in the above-mentioned clinics, were inspected from November 2002 to July 2003. Samples of soil and sand were taken from backyards, gardens, sandboxes, and playgrounds. There were 79 addresses from Mazowieckie Voivodship (a total 118 cases, among them 17 cases of ocular toxocarosis, 65 of visceral toxocarosis, and 36 of covert infection). The majority of children lived in rural areas (65.3%), fewer – in suburban regions (23.7%) and towns (11.0%). Till now, a total of 488 of soil and sand samples from 50 sites were examined with the use of Dada method (1979). Eggs of *Toxocara* spp. were found at 5 sites (10%). The present results confirm significance of environment pollution in spreading of toxocarosis in children. Low percentage of positive samples can be explained by chronic process of infection in humans and small chance to recover eggs in soil in spite of their long survival in the environment (up to 10 years).

Interviews carried out during the environmental study revealed insufficient knowledge of internists and ophtalmologists on toxocarosis as a potential zoonotic threat for children. It is important to disseminate basic knowledge on toxocarosis, possible sources of infection for children (puppies and kittens infected with *Toxocara* spp., polluted environment), and the methods of prevention.

SEQUENCE ANALYSIS OF ITS-2 rDNA REGION OF *TOXOCARA CANIS* AND *T. CATI* FROM POZNAŃ AREA

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Precise, specific identification of nematodes from superfamily *Ascaridoidea* in every stage of life cycle is necessary as a precondition for studying their life cycles, epidemiology, population biology and taxonomy. It is also indispensable for diagnostic aims and for undertaking the control measures. The genetics methods and especially PCR (Polymerase Chain Reaction) technique are very useful for the purpose. It allows to confirm results obtained using other methods, for example immunological techniques or observation. This study is focused on *Toxocara* – the nematode most frequent transmitted from pets to people. They are designed to help solving the problem, what is the percentage rate of two species – canine worm (*T. canis*), and the feline one (*T. cati*), in human toxocarosis.

To identify the parasites by PCR-based techniques, the most important mater is to choice of an appropriate target of DNA fragment. The nuclear ribosomal DNA (rDNA) region spanning the first (ITS-1) and the second (ITS-2) internal transcribed spacers are most useful in such molecular diagnosis. These sequences of individual species of nematode genomes are conservative but they are demonstrating great differences between species. Therefore sequences provide reliable genetic markers for identification of a broad range of ascaridoid nematodes to species. PCR technique with applying specific primers: T can 5' – AGTATGATGGGCGCGCCAAT – 3', N – 5' – TTAGTTTCTTTTCCTCCGCT – 3' and T cat 5' – GGAGAAGTAAGATCGTGGCACGCGT – 3' have been used for sequence analysis of two species: *Toxocara canis* (T can/N) and *T. cati* (T cat/N) originating from Poznań area. Amplified DNA fragments in PCR reaction were cloned to the plasmid vector p-GEM®-T easy and then sequencing was performed. The sequence data demonstrate 100% correspondence of *T. canis*

sequence examined to the sequence described by Jacobs (1997) and 98% of *T. cati* to the sequence of this species isolated by Zhu et al. (1998) in Malaysia. The sequence analysis confirmed that the species we examined were *T. canis* and *T. cati* and proved that applied primers could be valuable tools for identification of eggs isolated from soil during the environmental study in Poznań region.

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SOIL CONTAMINATION WITH *TOXOCARA* SPP. EGGS AND TOXOCAROSIS IN PEOPLE FROM RURAL AREAS OF POZNAŃ REGION (KOŁACZKOWO, LUSOWO)

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The degree of soil contamination with Toxocara spp. eggs and the prevalence of toxocarosis among inhabitants in Kołaczkowo and Lusowo villages were studied. In both examined places, 200 soil samples (each about 300 g) were collected from backyards, nursery- and preliminary school, playgrounds, parks, and streets. For detecting *Toxocara* spp. eggs 40 g mixed soil portions were examined in the laboratory by the flotation technique in saturated sodium nitrate ($NaNO_3$). Sera from 242 school children from Kołaczkowo and 105 adult people and 22 children from Lusowo were tested for specific anti-*Toxocara* antibodies using a commercial ELISA kit. An optical density (OD_{405}) equal or higher than 1200 was consider as seropositive. Additionally, clinical and epidemiological examinations of people were performed.

In the examined villages both, soil contamination with *Toxocara* spp. eggs and the prevalence of anti-*Toxocara* antibodies among examined people were high but there was no direct relationship between the two figures. In Kołaczkowo out of 200 soil samples examined 14.7% were positive and contained 152 *Toxocara* spp. eggs (106 were recognized as *T. canis*). There, out of 242 children examined 14.5% were infected. In Lusowo out of 200 soil samples examined 8.0% were positive and contained 103 eggs of *Toxocara* spp. (89 were recognized as *T. canis).* In this village, out of 127 people examined 30.7% were seropositive. Among 74 people with toxocarosis determined by positive serological test only asymptomatic cases were observed also in the foci with a high soil contamination. In the rural areas, the most contaminated places were backyards close to the household (21.7% and 16.3% samples with *Toxocara* spp. eggs). It is also worth to notice that infective eggs of *Toxocara* spp. were found on playgrounds of the nursery school (in Lusowo) and the primary school (in Kołaczkowo) and also in the area organized for roasting sausages on fire (in Lusowo).

The results indicate that apart from degree of soil contamination with the infective eggs some other factors are important in exposure of people for toxocarosis, for example age, hygiene, species of *Toxocara* (*T. canis* or *T. cati*), etc.

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MILK AS A POTENTIAL ROUTE OF *NEOSPORA CANINUM* TRANSMISSION

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Neospora caninum, an apicomplexan protozoan has a worldwide distribution. Domestic dogs are recognized as a definitive host but parasite was found as well in cattle, sheep, goats, horses, deer, monkey, foxes, coyotes. Although this coccidian has not been found in humans, some data revealed the presence of anti-*Neospora* antibodies.

This species was first isolated from a paralysed dog in 1984, and has recently gained considerable attention, due largely to its impact on the dairy and beef industry where it caused economic losses due to reproductive failure associated with abortion.

The parasite can be transmitted transplacentally in several hosts and the vertical route is the major mode of its transmission in cattle, perhaps for several generations. However, very low level of horizontal transmission (consumption of oocysts, placental membranes, or aborted bovine fetuses) have been taken into account.

Recently, it was demonstrated that culture-derived N. *caninum* tachyzoites added to milk were infectious to newborn calves when given by the oral route. The possibility of lactogenic infection, particularly by the practice of feeding pooled colostrum to newborn calves has been mentioned. However there is no evidence that feeding of milk which contained N. *caninum* tachyzoites may be the route for humans infection.

The aim of the study was to examine milk of seropositive cow for both, IgG level to antigens of N. *caninum* and the presence of DNA of the parasite. Additionally, the influence of some exogenous factors on the growth of tachyzoites of the reference strain of N. *caninum* (NC-1) and their penetration of Vero cells in an *in vitro* system.

PCR test performed with primers Np6 and Np21 gave a 328 bp product in examined samples and confirmed the presence of N. *caninum* DNA in milk of seropositive cow. Moreover, our results revealed that milk sample from a seropositive cow showed more than five times higher IgG level against N. *caninum* antigen when compared with a seronegative sample.

NC-1 tachyzoites were incubated in milk from seropositive and seronegative cows for 1 and 7 days at +4˚C. Only tachyzoites incubated one day in milk from seronegative cow grew and were able to invade a monolayer culture.

NC-1 tachyzoites were incubated also in PBS for 72 h, 7, 14 and 21 days at +4˚C. All incubated samples of tachyzoites transferred into monolayer culture system were alive, grew and invaded Vero cells.

Moreover, the influence of UV, freezing (- 20˚C), heating in microwave (+ 100˚C) and sterilization on the viability of tachyzoites were examined. Except UV, the results revealed that after the treatment, the tachyzoites were death and did not invade any Vero cells.

In summary our data confirmed that vertical transmission of N. *caninum* in herd *via* milk from seropositive cows is possible and therefore N. *caninum* transmission *via* milk in humans should be taken into account.

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CRYPTOSPORIDIUM PARVUM IN HIV PATIENTS FROM WROCŁAW

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Cryptosporidium parvum is a coccidial protozoan parasite known to cause enterocolitis and diarrhoea in humans, particularly in immunocompromised hosts. This study detect cryptosporidiosis in HIV patients treated in the Department of Infectious Diseases in Wrocław.

Methods: From January 2002 to June 2003, a total of 576 stool samples from 86 HIV-infected patients (20-42 years of age) were examined.

Diagnosis of cryptosporidiosis was made by detecting oocysts *C. parvum* in stool specimens with modified Ziehl-Neeelsen stain, carbol-methyl violet method and immunoenzymatic test (ProSpect Cryptosporidium Microplate Assay produced by Alexon Inc.). Intestinal biopsy specimens from one patient with diarrhoea were stained with 2% Giemsa.

Results: *C. parvum* was found in 8 HIV patients (9%).

Conclusions: *C. parvum* infection rate was similar to the rates reported by other Polish centres, but higher than of retrospective American series (5%).

ROLE OF MULTICENTRE EPIDEMIOLOGICAL AND CLINICAL INVESTIGATIONS IN THE DIAGNOSIS OF LIVER ALVEOLAR ECHINOCOCCOSIS IN POLAND

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The recent reports concerning an increasing incidence of *E. multilocularis* infection among red foxes in Poland, especially in the regions of Warmia, Mazury, Pomorze, and Podkarpacie, required an implementation of a nation-wide programme for the prevention and early detection of alveolar echinococcosis in humans. The aims of the collaborative study that involved the parasitological clinics from the university centres in Poznań, Gdynia, and Białystok were: (i) to determine the incidence of *E. multilocularis* infection in the human population of Poland and its correlation with previous results of veterinary investigations in foxes, (ii) to verify parasitologically the registered cases of suspected alveococcosis by specialized centres, and (iii) to standardize diagnostic and therapeutic procedures for this parasitic disease. The epidemiological and clinical studies were managed according to the uniform questionnaire elaborated by the European project coordinator (European Registry of Alveolar Echinococcosis) in Ulm (Germany). The evaluation of the parasitic spread and the developmental stages of the disease were based on the international classification system, including hepatic location of metacestode, extrahepatic involvement of neighbouring organs, infiltration of vessels and biliary tracts or presence of distant metastases as visualized by imaging techniques. The clinical diagnosis was based on typical imaging findings, immunodiagnostic tests with specific Em2plus and Em18 antigens, and confirmed by positive histopathological and/or molecular examinations. Since 1992, 23

cases of alveolar echinococcosis have been reported in Poland. The patients lived in the warmińsko-mazurskie, pomorskie, and lubuskie districts of Poland. Metacestodes were detected in the liver of all the cases, located in the both (n = 13) or in one of lobes (n = 10). In four persons, the parasite had spread to the adjacent organs or its growth was characterized by the distant metastasis formation in the lungs and brain; death occurred in 5 cases. The patients were treated by radical surgery with concomitant long-term intensive chemotherapy with albendazole. Conclusions: (i) Multicentre studies contributed to the successful detection of clinically-difficult cases of liver alveococcosis in the area of the whole country. (ii) Collaboration with veterinary and sanitary services, and physicians of various medical specialties is crucial for a more effective detection of alveococcosis in Poland. (iii) In all cases with irregular heterogenous masse in the liver suggesting tumor growth, shown by imaging techniques, *E. multilocularis* infection should necessarily be considered in the differential diagnosis of space-occupying lesions.

NEW METHOD FOR THE IDENTIFICATION OF *ECHINOCOCCUS MULTILOCULARIS*

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In 1993 Bowles & McManus proposed the JB11 and JB12 – primers specific for Platyhelminthes. They allowed to study more closely many species of parasites but also to use PCR for a detection of the presence of parasites (Cestoda and Trematoda) in a material sampled. Slight modification of this method presented here, allows for specific detection of *Echinococcus multilocularis*. This modification based on a primers JB11+75 i JB12 which amplify fragment of mtDNA could be more efficient than gene U1snRNA used so far. The analysis showed that this set of primers is specific for *E. multilocularis* and could be used to distinguish it from closely related *E. granulosous*. The new method is based on the use of the guanidine thiocyanate extraction buffer after evaporation of the ethanol from the tissues by drying (Tkacz & Pawlowski 1999) and PCR with the primers JB11+75 and JB12. Practical application of this method for the identification of *E. multilocularis* could increase the ability to diagnose and research of the alveolar hydatid disease in Poland, what is important considering the deadly consequences of the late diagnosis.

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THE INFLUENCE OF PHA-P ON A LEVEL OF APOPTOSIS AND NECROSIS IN THE SELECTED ORGANS OF MICE IN THE COURSE OF THE INFECTION WITH *TRICHINELLA SPIRALIS*

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The purpose of this experiment was determination of the level of apoptotic and necrotic lymphocytes in the spleen, lymphatic glands (axillary and mesenteric lymphatic nodes) and in the muscular

inflammatory infiltrations in the mice, which were treated with PHA-P before the infection, and in comparison with the mice, to which mitogen was not administrated.

Investigations were conducted on 42 CFW female mice, aged 3 months which. The mice were divided into three groups (Group I- the mice only infected with *T. spiralis*; Group II mice before the infection treated with PHA-P; Group III- healthy mice). PHA-P (Lecitin from *Phaseolus vulgaris* PHA-P, Sigma), dissolved in 0.9% NaCl was injected in a single dose of 10 mg/kg intravenously 24 h before infection with trichinellae. The mice were infected per os with a dose of 200 larvae *T. spiralis*/mouse; two mice of each group were killed on 7, 14, 21, 28, 35, 42, and 60 day post infection (dpi). During the section, fragments of the spleen, lymphatic glands (axillary and mesenteric lymphatic nodes) and muscles were collected, which were then crumbled mechanically and suspended in PBS. The suspensions were centrifuged on a layer of Ficoll/Uropolinum and the lymphocytes obtained were incubated using Annexin-V-Fluos Staining Kit (Boehringer Mannheim), which allowed for simultaneous recognition of both apoptotic and necrotic cells. The reaction was set according to the enclosed prescription and analysed using a FACS Calibur (Becton-Dickinson) flow cytometer.

In the control group (infected with the larvae of *T. spiralis*), the highest percentage of the apoptotic cells was found on 7 dpi in the spleen (27.9), on 21 dpi – in the axillary lymphatic nodes (31.2) and among the cells of the muscular inflammatory infiltration (44.5) and on 28 dpi – in the mesenteric lymphatic node (31,9). On the other hand, the highest level of the necrotic lymphocytes was found on 14 dpi in the axillary lymphatic node (59 %), in the mesenteric lymphatic nodes (56.7 %), in the cells of muscular inflammatory infiltration (15.6%) and in the spleen (34.1%).

In the group of the mice treated with PHA-P and infected with *T. spiralis*, the highest level of the apoptotic cells in all the examined organs occurred on 21 dpi: in the spleen 37%, in the axillary lymphatic nodes 30.2 %, in the mesenteric lymphatic nodes 33.2%, and among the cells of muscular inflammatory infiltration 46.2%. As far as the latter is concerned, another, nearly identical peak was found on 42 dpi. Different behaviour was observed in the necrotic lymphocytes, the highest level of which occurred on 14 dpi: in axillary lymphatic nodes (63.2%), in the mesenteric lymphatic nodes (65.3%), in the muscular inflammatory infiltration: (19.5%), and on 35 dpi – in the spleen (28.8%).

The foregoing findings should be estimated in comparison with the results obtained in healthy animals, where the highest level of the apoptotic lymphocytes reached up to 10.6% in the spleen, 8.5% in the axillary lymphatic nodes, and 8,2% in the mesenteric lymphatic node, whereas the level of the necrotic lymphocytes reached 20.6% in the spleen, 25.1% in the axillary lymphatic nodes, -and 20.2% in the mesenteric lymphatic nodes.

It is evident, from the results, mentioned above that:

(1) the level of both apoptotic and necrotic lymphocytes increased in both groups of the infected animals, however, it was a little higher in the mice treated with PHA-P;

(2) in the lymphatic organs of both groups of animals, maximum values were higher for the necrotic lymphocytes, contrary to the lymphocytes of the inflammatory infiltration, where there predominated distinctly a process of apoptosis, which, as it is known, is more favourable for a host.

BIOLOGICAL CONTROL OF PARASITES IN ENVIRONMENTAL PROTECTION

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Natural parasites (viruses and fungi) as well as pathogenic bacteria are used in the environmental friendly biological control of the arthropods – vectors of parasitic bacterial, viral (etc.) diseases and pests. In the international strategies of vector-borne diseases the microbial larvicides containing the

crystalliforous bacili of *Bacillus thuringiensis* and *B. sphaericus* (Vactobac, Bactimos) play the most important role. Also the fungi are important pathogens of many lepidopteran and coleopteran pests. However, only three species: *Beauveria bassianca*, *Metarhizium anisopliae* and *Verticillium lecanii* are used in field control. Specific association between some parasitic nematodes from the family of *Steinernematidae* and *Heterorhabditidae* and bacteria of *Xenorhabdus* genus can be included to microbiological control. Nematodes of *Heterorhabditis* and *Steinernema* genera are used for tick control: *Amblyomma americanum*, *Boophilus anulatus*, *Dermacentor variabilis*, *Hyalomma dromederi*, *H. excavatum*, *Ixodes scapularis*, *Rhipicephalus appendiculatus*, *R. bursa, R. evertsi*, *R. sanguineus*, *Argas persicus*, *Ornithodorus moubata*, *O. tholozani* – parasites and vector of diseases. Similar activity have entomopathogenic nematodes, mostly of the family Mermithidae, including some hundreds of obligatory insect parasites, rare other invertebrates. In this case, only parasitic larvae search for the hosts and cause their death. In field control of malaria vector the most important is *Romanomermis culicivorax*; its larvae are reared *in vitro* to the infective stage and the next are introduced to water bodies – habitats of mosquito larvae. Larvae of *Mesomermis flumenalis* are used in onchocercosis control. Nematodes are introduced to the streams – the place of *Simulium damnosum* development. Among the nematode parasites of pests is *Hexamermis albicans* (in length of 12 cm); its prevalence in potato beetle population can reach near 95%. Free-living entomopathogenic nematodes are *Heterotylenchus bovien* causing insect sterilization. They are used in biological control of flies-vectors of parasitic nematode *Parafilaria bovicola* locating in submucosa tissue of Equidae.

ENVIRONMENTAL CONDITIONS OF TRICHINELLOSIS

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Trichinellosis is a parasitic zoonosis caused by nematodes of the genus *Trichinella* which consists of eleven genotypes (eight of them are very well defined species and three have not been determined within taxonomical range yet). However, the genus *Trichinella* shows a cosmopolitan distribution, all the species are characterized for particular climate zones. It has been observed that different reservoirs, sources of infection and transmission patterns of the parasite depend on both the *Trichinella* species and the climatic zone. In addition, the parasite may be transmitted and maintained in the natural environment (sylvatic cycle) and synanthropic environment (domestic cycle) both among wild and domestic animals. However, although those environments play a role as separate reservoirs of the parasite they may also infiltrate each other and thus complicate the epidemiological process. It has been shown that environmental agents such as the freeze resistance of parasites, food availability in the environment and also the cannibalistic and scavenger behaviour of domestic and wild animals influence transmission and hosts infection. Human behaviour and its impact on natural ecosystems is also one of the most crucial environmental agents that affect parasite transmission. Improper disposal of meat offal, a low level of sanitation, and breeding standards on farms, local cultural practices among hunters and farmers as well as people migration are elements which affect transmission and the possibility of infection with *Trichinella*.

THE INFLUENCE OF THE FUNGAL METABOLITES FROM *CONIDIOBOLUS CORONATUS* ON THE *IN VITRO* CULTURES OF INSECT HEMOCYTES

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Among current methods of the biological control of insect pests, the use of soil entomopathogenic fungi carries lots of promises. Infection mechanism in the entomopathogenic fungi is a very complex process, which is not yet clear. This process involves both toxic substances (mycotoxins) and enzymes (proteases, chitinases and lipases secreted by fungi) which degrade insect cuticle. Each of these factors influences the pathogenicity of the fungus. We attempted to study the effect of fungal metabolites from *Conidiobolus coronatus* on the *in vitro* cultures of the hemocytes of three insect species – *Galleria mellonella*, *Dendrolimus pini*, and *Calliphora erythrocephala*. After addition of fungal metabolites to the *in vitro* cultures of hemocytes, deformations of the plasmatocytes, oenocytes, and granulocytes were observed. In all insect species oenocytes became more irregular and degranulation of granulocytes was increased. The adhesion to the surface was also affected in case of plasmatocytes of *D. pini* and *G. mellonella*. Proteins from the *C. coronatus* post-incubation filtrates were precipitated with the TCA, dialyzed against water and subsequently separated using polyacrylamide gel electrophoresis (SDS-PAGE). After staining by Coomassie blue we determined their respective masses using the molecular weight marker. We found 15 polypeptydes in the post-incubation medium. The particles of highest concentration ranged from 28-kD to 52-kD. Further research is needed to answer the question which metabolites secreted by the fungus *C. coronatus* are responsible for the induction of the changes in morphology and behaviour of hemocytes.

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PARASITES OF BREAM (ABRAMIS BRAMA) AS INDICATORS OF THE ENVIRONMENT QUALITY

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For many years fish parasites have been considered to be a good indicator of the quality of water environment. Apart from individual species, whole parasite communities may be used for such purpose. In order to identify which communities and which parameters are most useful as bioindicators of the quality of freshwater environment, 4 communities and 2 guilds of the parasites of bream *Abramis brama* were examined. Additionally, both infracommunities (infraguilds) and components communities (component guilds) were analysed.

Structure of parasite associations was described by its richness, diversity, and dominance. The following communities and guilds were examined: (i) intestinal parasites, (ii) gill parasites, (iii) adult forms of autogenic parasites, (iv) all metazoan parasites, (v) eyeflukes, (vi) small monogeneas.

The most important factor that influences majority of analysed indicators is seasonality. Moreover, other factors – like type and location of reservoir – need to be taken into account.

The communities which are most suitable to be used as a bioindicators of water quality are intestinal parasites and gill parasites of bream.

Detailed results are presented in a PhD Thesis [The structure of some parasite communities of bream (*Abramis brama*) in relation to the type of water body and the pollution]; W Stefanski Institute of Parasitology PAS, Warszawa 1999.

CHANGES IN THE PARASITE FAUNA OF ROACH, *RUTILUS RUTILUS* (L.) IN SELECTED LAKES OF THE MASURIAN LAKE DISTRICT

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A lot of changes have taken place in the parasite fauna of roach in lakes Dgał Wielki and Warniak, after over 20 years that have elapsed since the last survey (Grabda-Kazubska et al. 1978), which was carried out during a re-stocking of those bodies of water. Despite of the absence of some, previously recorded parasites, the parasite fauna was enriched by 17 additional species, representing both direct or complex life cycles. The direct manipulation of qualitative and quantitative composition of the ichthyofauna of those lakes, in the 1970s and the 1980s, resulted in drastic changes of the entire biocoenosis, including invertebrates and vertebrates, known as potential hosts for parasites. Nesting and feeding areas of birds were also limited and the birds themselves were additionally threatened by a high-density local population of American mink.

New (compared to the period 1978-1984) findings of metacercariae and adult specimens of digenean flukes, two acanthocephalan species, and the increased prevalence of the tapeworm *Paradilepis scolecina* and the nematode *Rhapidascaris acus*, can be explained by a gradual reconstruction of the biodiversity of the flora and fauna and the increase of absolute numbers of invertebrates, particularly planktonic crustaceans and benthic oligochaetes (Zdanowski 1999). The absence of the tapeworm *Ligula intestinalis* and a drastic decline of the nematode *Philometra ovata* infections in roach may be a good evidence for the absence of previously recorded invertebrates.

MICROSPORIDIOSES OF AQUATIC INVERTEBRATES IN WATER RESERVOIRS OF DIFFERENT TYPES

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Microsporidioses cause serious problems in aquaculture of crayfish and shrimp, and microsporidians are recognized as parasites of considerable importance in fisheries and continue to gain increasing importance in veterinary and human medicine. The surveys carried out during 1986-1998 covered a number of reservoirs in the Dnepr and Danube basins of Ukraine and different water-bodies in the northeast of Poland.

The data on five microsporidians infecting 14 amphipod hosts has been collected in the Dnepr and Danube estuaries, the reservoirs of the Dnepr, and the river Tisa and Dniester. Among them, microsporidium *Thelohania muelleri* was registered in 9 host species, belonging to ancient-freshwater, Ponto-Caspian and Mediterranean faunal complexes. A cluster analysis of morphometrical characters has shown the existence of several morphometrical groups within populations of *T. muelleri*, corresponding to the variable salinity of the environment. Tetrasporous microsporidium *Curleya orchestiae* parasitising crustaceans *Orchestia montagui* and *O. bottae*, was recorded only in the salt-water regions of the estuaries of Dnepr and Dniester, and the Jagorlytski Bay of Black Sea. Microsporidian parasites *Nosema dikerogammari* and *N. pontogammari* were distributed geographically as their hosts – *Dikerogammarus villosus*, *D. haemobaphes*, *Pontogammarus crassus*, *P. obesus*, and *Chaetogammarus ischnus*, without valid morphometrical distinctions.

As a result of parasitological researches of freshwater invertebrates inhabiting Mazurian Lakes District of Poland it has been established, that Microsporidia infect mostly crustaceans (Daphniidae, Cyclopidae, Gammaridae) and migde larvae (Chironomidae). Microsporidioses of water invertebrates were registered mainly in the small reservoirs that were polluted with organic pollutants. In mesotrophic water bodies Microsporidia were rare and prevalence of infection was established at about 1.5%.

Intracellular parasitism is a profound specialized form of relationships between the members of the biocoenotic pair, presented by the host-parasite complex and its environment. The mechanism of such a relation is finely adjusted in Microsporidia, the most ancient representatives of parasitic eukaryotes.

THE COMMUNITIES OF BLOOD PARASITES IN FIELD-MOUSE *APODEMUS AGRARIUS*

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The field mouse *Apodemus agrarius* plays a great role as a zoonotic reservoir of zoonoses in non-wooded areas, such as urban and rural environments (Pruszyńska 1988, Bull. Inst. Mar. Trop. Med. Gdynia, 39, 91-107). However, the ecological aspects of infection of *A. agrarius* with blood parasites, are not very well known.

The investigations of infection of *A. agrarius* with blood parasites were carried on in environments changed by human activity in Poland (Katowice agglomeration, Kosewo near Mikołajki, Mazurian District) and the East Slovakia (Košice agglomeration, Zemplínske Hradište, Trebišov, Boťany). The catches were done using choker and alive traps. Blood smears were made, stained with Giemsa and examined under light microscope. The following blood parasites were found: *Trypanosoma grosi*, *Babesia microti*, *Hepatozoon* sp. and *Bartonella* sp. A relatively low prevalence of blood parasites was recorded in *A. agrarius* in comparison with other *Apodemus* mice. These results correlate with infestation with blood-sucking arthropods. The infection of *A. flavicollis* with fleas and ticks is higher when compared to *A. agrarius* (Stanko and Miklišová, In: Stawonogi w medycynie, 2002 (Eds.) A. Buczek, Cz. Błaszak, 105).

DIVERSITY OF HELMINTH SPECIES IN POPULATIONS OF WILD RODENTS, AND THEIR ROLE IN MAINTAINING THE ZOONOTIC RESERVOIR OF *CRYPTOSPORIDIUM PARVUM*

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Many domestic and wild animals are the source of the parasitic protozoa – *Cryptosporidium parvum*, causing cryptosporidiosis, which is a dangerous human disease. This parasite attacks the alimentary and respiratory mucosae of vertebrates, including humans. It has a direct life-cycle, thus both the sexual and non-sexual cycles occur in the same host. The infective stages are oocysts, which are released in high quantities into the environment via the faeces of infected animals. The parasite can be contracted orally or through contact with infected faeces.

Analysis of both single and coinfections found that single infections are less intensive and shorter. It is important to know the mechanisms involved in nematode infections, as these can cause hosts to be predisposed to further parasite infections. This situation is often found in wild rodents, where coinfections occur between protozoans and helminths. This coinfection can cause the immunity of wild rodents to break down, leading to massive parasite reproduction.

Furthermore, researchers from the Mazury Lake District region have shown, from three different sites, a significant difference in both the intensity of infection of *C. parvum* and in the size of helminths diversity in rodents.

We were comparing helminths of *Clethrionomys glareolus* (bank vole) from three different sites. Eight species of helminths were identified, six of which were nematodes, and three tapeworms.

Although the three sites being examined had similar ecological structures, there was a significant variation in the parasite community at each site. In two of the sites the most common parasite identified was *Heligmosomum mixtum* (prevalence 95 and 44.9%), however it was absent at the third site. In contrast, the prevalence of *Heligmosomoides glareoli* was very low at sites 1 and 2 (7.5 and 2.4%), but was high at site 3 (79.3%). However, *C. parvum* was identified at all three sites. High prevalence and intensity of infection of these protozoans in bank voles can be caused by concurrent nematode infections, due to a reduction in host immunity. Moreover, seasonal changes in nematode prevalence and intensity, with high prevalence in late summer can also have the influence for *Cryptosporidium* infections.

HORIZONTAL TRANSMISSION OF *B. BURGDORFERI* S.L AMONG WILD RODENTS

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The zoonotic reservoir of *B. burgdorferi* s.l. constitute ticks of the *Ixodes persulcatus* „complex" groups, and *I. ricinus* in Poland. The ticks infect more than 300 species of reptiles, birds, and mammals. It is known that not all these animals are hosts of ticks and are source of *Borrelia* infections. The circulation of *B. burgdorferi* in the natural environment and the prevalence of infections among

humans depend on the high density of the right vector and the presence of animals capable of transmitting spirochetes.

For the first time in Poland, a complex study was conducted, simultaneously taking into account the environmental conditions of *B. burgdorferi* transmission and the relationships between vector, host and the prevalence of spirochetes. The research area was characterized in terms of the density of *I. ricinus* and its potential hosts.

The survey conducted from 1998 to 2001 facilitated the recognition of transmission of spirochetes. The research materials constituted 10 938 *I. ricinus* ticks and 1305 rodents from three species: *A. flavicollis*, *C. glareolus*, and *M. arvalis*. Immunofluorescent antibody assay (IFA) and the polymerase chain reaction (PCR) were used to examination *Borrelia* infections among ticks and rodents. The pathogens for human genospecies *B. afzelii* and *B. garinii* were differentiated in DNA probes isolated from ticks. The highest level of prevalence and intensity of rodent infections by immature development stages of *I. ricinus* proven in *A. flavicollis* population, were 92% and 11.6 ticks/animal, respectively. Those parameters were lower for *C. glareolus*, amounting to 77% and 6 tick/animal, respectively and the lowest for *M. arvalis* (35% and 5 tick/animal). The presence of *B. burgdorferi* was determined among 7% immature development stages of questing ticks and 9% among feeding ticks. The infection rate of yellow necked mice was 4.3% while in bank voles it was 1.7%.

The environmental and laboratory examinations demonstrate the significant role of rodents as a source of *B. burgdorferi* infection. The high abundance of rodents in the research area, and the high level of infection by ticks show that they are basic host species for ticks.

The presence of spirochetes among rodents and a higher level of spirochete infection among ticks parasites on the rodents in comparison with ticks found on plants point to rodents as the zoonotic reservoir and source of *B. burgdorferi* infection. The prevalence of *B. afzelii* and *B. garinii* in the research area can have a large practical significance in the epidemiology of tick-borne diseases.

This research was supported by the Foundation for Polish Science with a scholarship for young scientists.

INCIDENCE OF *BORRELIA BURGDORFERI* S.L. IN MOSQUITOES (CULCIDAE) COLLECTED IN THE ZACHODNIOPOMORSKIE PROVINCE

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The aim of the present study was to determine the infection level of mosquitoes with spirochetes *Borrelia burgdorferi* s.l. in forest areas of the city of Szczecin and its vicinity. Five sampling sites were selected in wooded areas associated with bodies of water. Host-seeking mosquitoes were lured by people collecting them. The sampling lasted from May to July 2003. The spirochetes, *Borrelia burgdorferi* s.l. present in mosquitoes were detected through indirect immunofluorescence assay (IFA) using rabbit anti-*Borrelia burgdorferi* antibodies and goat anti-rabbit IgG marked with fluorescein isocyanate (FITC).

A total of 712 females and 57 males were collected. They represented genera *Aedes* (71%) and *Culex* (29%). The infection level of the mosquitoes from the area studied amounted to 1.7%.

The results of the present study confirm a potential of those arthropods to spread Lyme borreliosis.

THE ECTOPARASITES OF SMALL RODENTS FROM LOWER SILESIA AREA

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The rodents were trapped in two habitats: the Wołów area fields (2000 year) and the forests of Masyw Ślęży (2002). There were collected 22 *Apodemus agrarius* (Pall.) from Wołów area and 19 rodents representing 4 species: *Apodemus agrarius* (4), *A. sylvaticus* (Linn.) (4), *A. flavicollis* (Melch.) (1) and *Clethrionomys glareolus* (Schreb.) (10) from the Ślęża Mountain complex (Masyw Ślęży). The rodents were examined for the presence of ectoparasites. There were found 3 species of lice (Anoplura): *Hoplopleura affinis* (Burm.), *H. acanthopus* (Burm.) and *Polyplax serrata* (Burm.), 5 species of fleas (Siphonaptera): *Ctenophthalmus* (*Ctenophthalmus*) *agyrtes* (Hell.), *Hystrichopsylla talpae* (Curt.), *Megabothris turbidus* (Rothsch.), *Leptopsylla segnis* (Schőnh.), *Peromyscopsylla silvatica* (Mein.) and 2 species of mites (Acari): *Laelaps pavlovskyi* (Zachv.), *Ixodes ricinus* (Linn). 54.5% of all collected rodents were found to be infected in the Wołów area and 84.2% in the Masyw Ślęży, respectively. The prevalence of lice infection was 45.5%, 13.6% – fleas and 9.1% – mites in the Wołów area. The prevalence of lice infection was 10.5%, 26.3% – fleas and 78.9% – mites in the Masyw Ślęży. The most dominant mite species in rodents from the Masyw Ślęży was *I. ricinus* (prevalence 73.7%). *I. ricinus* was absent in the Wołów area. *L. pavlovskyi* was found in both habitats (the prevalence in Wołów area was 9.1% and in the Masyw Ślęży 10.5%). Four species of fleas were collected from rodents in the Wołów area and only 2 in the Masyw Ślęży. *P. silvatica* was found only in the Masyw Ślęży and *C. (C.) agyrtes* were found in both areas. Lice *H. affinis* and *P. serrata* were found on *Apodemus agrarius*; *H. acanthopus*, and *P. serrata* on *Clethrionomys glareolus*. The highest number of ectoparasites occurred in single rodent in the Masyw Ślęży (*A. sylvaticus*) was 82 and in the Wołów area (*A. agrarius*) 13.

THE RESULTS OF EXAMINATIONS OF INTESTINAL PARASITES OF WOLVES (*CANIS LUPUS L.*) FROM BESKID ŻYWIECKI AREA – A PRELIMINARY STUDY

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The knowledge about parasites of animals, occupying the highest links of food chains, is still very poor and fragmentary. In case of native carnivorous mammals, no parasitological investigations were carried out because of protective status of these animals. Consequently, we have had all data proceeding from coprological examinations. Review of Polish parasitological literature shows that researches on intestinal parasites of free living wolves were carried out so far only by Frumaga and Wysocki (1949), Sołtys (1964), and lately by Kloch (2002).

Material for coprological examinations has been collected since March 2002 from Beskid Żywiecki area and it is going to finish at the turn of 2003/2004. The samples were preserved in 4% formalin then washed and examined by decantation technique.

As the result of 24 investigated samples (19 positive), we detected and identified eggs of parasites as follows: trematode *Mesostephanus* sp.? (1 egg in one sample out of 24 examined) and nematodes *Trichuris vulpis* (1/24; single egg), *Toxascaris leonina* (5/24; 1-4 eggs in a sample), *Toxocara canis* (14/24; 2-9), *Ancylostoma caninum* (11/24; 1-7), and *Uncinaria stenocephala* (1/24; 1). In addition we have found many excreted parts of strobila of a tapeworm; because of the lack of scolices we classified it to *Taenia* genus. Some of these identifications should be verified.

GNATHOSOME MORPHOMETRIC FEATURES OF *DERMACENTOR RETICULATUS* (FABR.) AND *DERMACENTOR MARGINATUS* (SULZER) LARVAE (ACARI: IXODIDAE) FROM SLOVAKIAN POPULATIONS

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In Poland only two [i.e. *Dermacentor reticulatus* (Fabricius, 1794) and *Dermacentor marginatus* (Sulzer, 1776)] of over 30 species of the genus *Dermacentor* are known.

The aim of our study was to compare gnathosome morphometric features of *D. reticulatus* and *D. marginatus* larvae from Slovakian populations. In our paper we considered five features: the length and width of gnathosome, length and width of hypostome, and palp length. The examined parameters have a significance in the determination of the larval stage of ticks.

For the study we used larvae reared in optimal laboratory conditions from adult ticks collected in the nature of Slovakia. The larvae were prepared and mounted in Four's fluid for microscopic examination. In order to examine the considered features of gnatosome 86 larvae of *D. reticulatus* and 67 larvae of *D. marginatus* were observed under the microscope. Besides two indices (width to length of gnathosome and width to length of hypostome) were calculated. Statistical analysis of the results was done with the use of computer program Statistica.

The results of our study are shown in the Table. The length and the width of gnathosome were significantly higher in *D. reticulatus* larvae. There was also a significant difference in the hypostome width between *D. reticulatus* and *D. marginatus* larvae. Among calculated indices only hypostome width to length index exhibited a significant difference between both species of ticks. Hypostome in both larval ticks is clavate with trunk dentition 2/2. Distinctive auriculae, triangle in the shape occur in *D. reticulatus* larva. In *D. marginatus* larva, auriculae are in the shape of acute-angled triangle.

Table. Gnathosome morphometric features of *D. reticulatus* i *D. marginatus* larvae from Slovakian populations

Feature	*D. reticulatus*					*D. marginatus*				
	mean	SD	min	max	n	mean	SD	min	max	n
Gnathosome length (µm)	17.6*	0.6	15.6	19.1	85	17.3*	0.9	15.3	19.1	66
Gnathosome width (µm)	14.5**	0.6	13.1	15.8	86	14.2**	0.5	13.4	14.9	66
Hypostome length (µm)	7.1	0.4	6.2	8.2	82	7.1	0.5	5.7	8.2	66
Hypostome width (µm)	4.0**	0.2	3.5	5.0	86	3.6**	0.2	3.2	4.2	67
Palp length (µm)	10.0	0.3	9.4	11.4	86	10.0	0.3	9.4	11.1	67
Gnathosome width to length index	0.83	0.04	0.7	10.0	85	0.81	0.11	0.7	0.9	66
Hypostome width to length index	0.56**	0.04	0.5	0.7	82	0.51**	0.04	0.4	0.7	66

*p<=0.05, **p<=0.001

THE INFLUENCE OF TEMPERATURE CHANGES ON *DERMACENTOR RETICULATUS* (FABR.) EGGS DEVELOPMENT

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Dermacentor reticulatus (Fabr.) is one of the most common tick species in Poland. The knowledge of biotic and abiotic factors influencing the development of ticks is of great importance in limiting this dangerous parasite of animals and humans. The aim of our study was to evaluate the influence of temperature fluctuations on development of eggs of *D. reticulatus*.

For the study we used eggs laid in laboratory conditions in optimal temperature and humidity, i.e. 25˚C, 90% r. h. Laid eggs were divided into four groups; each group included three daily laid egg batches. The control group was maintained continuously in optimal temperature until larvae hatching. The eggs of three examined groups were maintained in optimal temperature for 9 (group I), 6 (group II) or 3 (group III) days and then put into fluctuating temperature conditions (48 hours periodicity, 24 hours in 37˚C and 24 hours in 5˚C). The number of egg batches with at least one larva hatched, and duration of embryogenesis were registered. Statistical comparison of mean duration of embryogenesis was performed with the use of Kruskal-Wallis non-parametric ANOVA and U Mann-Whitney tests among control group, group I, and II. Group III was excluded from statistical analysis due to the number of egg batches.

Larvae hatching in all examined egg batches occurred in control group, in group I and II. On the other hand, there were no larvae hatching in two of three examined egg batches in group III. The duration of embryogenesis was 13.3 days in control group, 14.7 days in group I, 15.0 days in group II, and 16.0 days in group III. According to Kruskal-Wallis ANOVA mean duration of embryogenesis differed significantly ($p<0.1$) among control group, group I, and II. U Mann-Whitney test exhibited statistical difference in embryogenesis duration only between control group and group II ($p<0.05$).

The results of our study may indicate that temperature fluctuations of high amplitude, possible in our climate conditions in spring and autumn, cause disturbances in the development of eggs of *D. reticulatus*.

LOSS OF WEIGHT OF *ARGAS REFLEXUS* (FABR.) (IXODIDA: ARGASIDAE) FEMALES DURING OVIPOSITON

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Argas reflexus (Fabr.) is an ectoparasite of pigeons. Pigeon ticks, however, can harm people when their natural hosts are absent. Adult individuals of *A. reflexus* feed about 0.5 to 2 hours during the night.

In this study 18 females of *A. reflexus* collected from their natural habitat in Upper Silesia were used. They were fed on pigeons in the laboratory. After the feeding every female was weighed and subsequently held under optimal conditions, i.e. 25˚C and relative humidity of 30%, attained by using $CaCl_2$ solution. The measurement of the weight was repeated on the 53-day (during oviposition) and 102 day (after oviposition) of the experiment. The results were statistically worked out.

During 53 days after feeding females lost from 30.3% to 75.72% (mean 55.01 ± 12.19) of their weight just after feeding. The measurement on the 102 day after feeding showed that females' loss of weight progressed gradually. The weight of *A. reflexus* females on 102 day of the study was 46.64% to 18.02% (mean 28.74 ± 9.13) of the value at the beginning of the experiment. The duration of oviposition of *A. reflexus* females is 9-54 (mean 27.87 ± 12) days. During this period females lay 51-192 (mean 113.67 ± 39.28) eggs in 4-11 (mean 6.87 ± 2.33) egg batches.

The results of the study showed that the weight of *A. reflexus* females decreased gradually after the feeding. During this period oviposition occurs and the energy from the meal is exploited to produce and develop eggs.