

Review articles

Parasitic diseases and fungal infections – their increasing importance in medicine¹

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ABSTRACT. Basing on 43 lectures and reports from the scope of current parasitological and mycological issues presented during the 50th Jubilee Clinical Day of Medical Parasitology (Lodz, 19–20 May 2011), the increasing importance of parasitic diseases and mycoses in medicine was presented. Difficulties in diagnosis and treatment of both imported parasitoses (malaria, intestinal amoebiasis, mansonelliasis) and native parasitoses (toxoplasmosis, toxocariasis, CNS cysticercosis), as well as parasitic invasions coexisting with HIV infection (microsporidiosis) have been emphasized. The possibility of human parasites transmission by vertical route and transfusion has been discussed. The important issue of diagnostic problems in intestinal parasitoses has been addressed, noting the increasing use of immunoenzymatic methods which frequently give false positive results. It was highlighted that coproscopic study is still the reference method for detecting parasitic intestinal infections. The mechanism of the immune reaction induced by intestinal nematodes resulting in, among others, inhibition of the host innate and acquired response was presented. Mycological topics included characteristics of various clinical forms of mycoses (central nervous system, oral cavity and pharynx, paranasal sinuses, nails and skin), still existing problem of antimicrobial susceptibility of fungal strains, diagnostic and therapeutic difficulties of zoonotic mycoses and the importance of environmental factors in pathogenesis of mycosis.

Key words: medical parasitology, mycology, parasitoses, mycoses

Basing on lectures and reports delivered during 50. Clinical Day of Medical Parasitology (Lodz, 19–20 May 2011) the review of actual problems of medical parasitology and mycology is presented.

Archeoparasitology appeared as a new branch of parasitology in the beginning of the 20th century, when a new technique to rehydrate desiccated tissues allowed the finding of helminth eggs in mummies (A.C. Majewska, Medical University of Poznan). Archeoparasitology studies provided important information on human and animal parasitic infections, as well as on social and dietary behaviors in the past. Various species of parasites have been found in mummies as well as in animal and human fecal material from historic and prehistoric times in different part of the world. The earliest documented archeoparasitological discovery made by Ruffer in 1910 concerned the

detection of calcified *Schistosoma haematobium* eggs in the kidneys of Egyptian mummies of the twentieth dynasty (1250–1000 BC) [1]. Intensive study of coprolites and mummified corpses in search of developmental forms of human parasites were carried out in the years 1960–1980. In 1979 Ferreira et al. [2] introduced a new name for a subfield of archaeology-paleoparasitology. Application of the new research techniques (electron microscopy, immunoassays) since 1980, and molecular biology methods (PCR) since 1997 significantly facilitated the identification of the detected parasitological material. In many cases, it is difficult to identify parasites from archaeological material with the routine procedure, and then it is necessary to use immunological and molecular methods. Molecular characterization of ancient parasite DNA is very useful in making definitive

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diagnosis of ancient parasites and identifying genetic strains of single species. Immunological tests helped to identify cysts and/or coproantigens of intestinal protozoa *Giardia* spp. (Peru, 4300 years ago), *Entamoeba histolytica* (Switzerland, 5300 years ago), *Cryptosporidium* spp. (the Andes, 3000 years ago) in coprolites [3]. The DNA of *Trypanosoma cruzi* was detected in 39% of samples from 18 Chinchorro people mummies dating from before 9000 years found in the Atacama desert (southern Peru, northern Chile) [4]. The oldest eggs of intestinal parasites in coprolites were detected from before tens of thousands of years ago (8450–30000 years – *Trichuris trichiura*) [5]. Eggs of *Ascaris lumbricoides* originating from the „Great Cave” in France dating 30000 years allowed to establish that the primary species of the genus *Ascaris* was a human, not swine worm, because domestication of wild pigs took place only about 9000 years ago [6]. Recently, study of human coprolites was performed in Poland; the aim of this study was recovery of helminth eggs and *Giardia* cysts from archeological excavations of latrines, dating from the 14th to the 17th centuries, in Poznan [7]. In examined samples, *Trichuris* and *Ascaris* eggs were most frequently identified, less often *Opisthorchis* sp., *Diphyllobothrium* sp., *Taenia* sp. and *Syphacia* sp. The presence of few *Giardia* cysts were observed in examined latrine samples from the 17th century [7].

Toxoplasmosis and toxocarosis are the most common antropozoonoses acquired *per os*, and still pose a valid and serious epidemiological problem (H. Mizgajska-Wiktor, W. Jarosz, R. Fogt-Wyrwas – Physical Education Academy in Poznan). *Toxoplasma gondii* is a common, global protozoan parasite, which requires both a definitive host and an intermediate host to complete its life cycle. Although felines are the only definitive host of *T. gondii*, any warm-blooded animal, including humans, can be infected. *Toxocara canis* and *T. cati* are common ascarid parasites of dogs and cats, and are considered to be the principal etiological agents of visceral larva migrans syndrome (VLM). *T. gondii* oocysts after 1–5 days presence in the external environment reach the stage of invasiveness; the intake of 10 oocysts is sufficient to infect intermediate hosts, and 100 of the protozoan oocysts for definitive host. By contrast, eggs of *Toxocara* spp. acquire invasiveness after 10–20 days of staying in the soil, but only one invasive egg can cause invasion in the host [8]. The

developmental forms above parasites have different time vitality in the soil (*T. gondii* oocysts – about 2 years; *Toxocara* eggs – a few years). Children more frequently have clinical symptoms of these parasitoses because of the closer contact with contaminated soil in yards and sandpits, the lack of hygiene, and because of eating dirt. Similar location of parasites in the human organs is detected in the course of toxoplasmosis and toxocarosis (central nervous system, skeletal muscles, heart muscle, eyeball, lungs, liver, and kidneys). *Toxocara* larval migration in the body can cause various clinical syndromes; visceral larva migrans (VLM), ocular larva migrans (OLM), neurotoxocarosis and asymptomatic toxocarosis are described. The first evidence for the existence of different strains of *T. gondii* came from isoenzyme analysis of isolates mainly of French origin [9]. Parasite strains can be divided into three clonal lineages: types I, II, and III, according to their pathogenicity in mice. Type I strains are uniformly lethal and have a greater a capacity to cross tissue barriers *in vitro* and *in vivo*, whereas types II (intermediate virulence) and III (low virulence) show much lower levels of pathogenicity. Congenital toxoplasmosis is associated mainly with type I and II strains. Most strains from patients with acquired immunodeficiency syndrome are type II, and animal strains are predominantly type III. A number of issues concerning the epidemiology of invasion with *T. gondii* and *Toxocara* spp. remain inadequately explained. Current research focuses, among others, on attempts: to determine the prevalence of *T. gondii* in different soil types (clonal lines I, II and III), to detect the main source of *Toxoplasma* invasion in children (contaminated soil, or food?), to compare the degree and dynamics of soil contamination with *T. gondii* oocysts in urban and rural environments, to determine the role of synanthropic foxes in contamination of the environment with *Toxocara* eggs. The following difficult questions pose a challenge to the researchers: whether genetic types or subtypes are organ-specific? whether different genotypes of the parasite have different opportunities of the host colonization? whether there is genetic variation in *Toxocara* and *T. gondii* depending on geographical location? how proteins and gene expression of these parasites modify in response to changes in the environment?

Parasitic infection in pregnant women may affect pregnancy (J. Błaszowska, Medical University of

Lodz). Many premature births are recorded in the course of parasitoses (malaria, echinococcosis, ascariasis, amoebiasis), retarded fetal growth, and even abortion (malaria, toxoplasmosis, Chagas disease). Vertical infection is the consequence of transmission of the parasite from mother to offspring: through placenta (*Toxoplasma gondii*), during childbirth (*Trichomonas vaginalis*), via lactation (*Ancylostoma duodenale*). Congenital toxoplasmosis is frequently recorded in the offspring of mothers who become infected with *T. gondii* during pregnancy [10]. However, reactivation of invasion during pregnancy was noted, most often in mothers whose CD4+ lymphocyte count falls below 200/ml. In addition, there were reported cases of superinvasion with another *T. gondii* strain, more virulent than the primary, in seropositive immunocompetent pregnant women. The possibility of reinvasion and superinvasion of *T. gondii* in pregnant women undermines the current belief that the immunity produced in the course of toxoplasmosis protects the host for life from reinfection [11,12]. Congenital visceral leishmaniasis may also be a consequence of both, the primary invasion of *Leishmania* during pregnancy, and reactivation of latent leishmaniasis [13]. Congenital malaria occurs mostly as a consequence of trophoblast damage, placental immaturity and leakage, premature rupture of membranes [14]. There were described intrauterine fetal infection with microfilaria (*Wuchereria bancrofti*, *Onchocerca volvulus*, *Mansonella perstans*) and larvae (*Trichinella* sp., *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus*, *Toxocara canis*, *Strongyloides* sp.). It is possible breast-feeding transmission of protozoa (*Trypanosoma*, *Leishmania*) and nematode larvae (*A. duodenale*, *N. americanus*, *Strongyloides* sp.) to the neonate. Data from literature show that congenital parasitoses are most frequently recorded as a consequence of infecting women during first pregnancy with blood and tissues parasites (*Plasmodium*, *Toxoplasma*, *Leishmania*). Difficulties with proper diagnosis and the lack of invasive factors identification in aborted fetuses and stillbirths (*Toxoplasma*, *Leishmania*, *Trichinella*) lower the value of congenital parasitoses prevalence.

Gastrointestinal parasite infections provide a unique challenge to the host immune system (M. Doligalska, University of Warsaw). Parasitic nematodes in the host alimentary tract can colonize the intestine without prior migration in the host or-

ganism (*T. trichiura*, *E. vermicularis*, *Heligmosomoides polygyrus*) or after migration (*A. lumbricoides*, *N. americanus*, *A. duodenale*, *N. brasiliensis*). However, regardless of this fact, the common features in the immune response induced by intestinal nematodes are high activity of Th2 lymphocytes and inhibition of the immune response. The classical reaction of the host to helminth infections is the induction of Th2 immune responses with a regulatory component. Dendritic cells (DC), as central players in the induction and maintenance of immune responses, play a prominent role in both these processes [15]. Helminth-derived molecules can directly interact with DC to condition them to drive Th2 or Treg responses. Additionally, innate cells (e.g. mast cells, macrophages, granulocytes), tissue cells (e.g. epithelial cells) and adaptive immune cells (e.g. B cells) secrete several cytokines and other factors in response to helminth infections that can act on DC to shape their immune polarizing properties. The presence of intestinal helminths in the host leads to inhibition of host innate and acquired responses, expansion of CD4+CD25+ regulatory lymphocytes, immunosuppressive activity independent of cytokines with the participation of CD8+ lymphocytes. An important mechanism of immunoregulation induced by intestinal nematodes is the impact on apoptosis of host cells that cause elimination of some of the immunologically competent cells subpopulations (CD4+ auxiliary lymphocytes) [16]. Epidemiological studies show that poor hygiene and ineffective antiparasitic prophylaxis among poor societies facilitate long-term persistence of intestinal nematode infections. Simultaneously, there are cases of nonspecific inflammatory bowel disease and asthma, increasingly reported in industrialized countries. The inverse correlation between the incidence of allergies and intestinal parasitoses was the basis for launching a „hygiene hypothesis”, whereby the rarer helminth infections of children in the period of immune system maturation may condition increased incidence of lifestyle diseases later in life. Currently, the results of both epidemiological and experimental studies suggest that in the course of certain diseases the helminth invasion may inhibit gastrointestinal inflammation. There were noted beneficial effects of *Trichuris suis* invasion in people with colitis, Crohn's disease, or of *T. spiralis* in type I diabetes. There were also demonstrated inhibition of gastrointestinal inflammation in model invasions in laboratory animals experimentally

infected with *T. spiralis* or *Heligmosomoides polygyrus* [17], and alleviating allergy symptoms in experimental infections with *Nippostrongylus brasiliensis* or *H. polygyrus* [18].

The success of an epidemiological program against infectious diseases depends on an effective prophylactic vaccine (H. Długońska, M. Grzybowski – Lodz University). The effect of vaccination depends on many factors such as sex, age, present hormonal status, as well as the composition of microbiota [19]. Studies in humans and experimental animals indicate that the number and activity of immune cells (e.g. antigen-presenting cells, APC) and the severity of inflammatory reactions are higher in females. There were found stronger stimulation of the immune system and more frequent side effects in the majority of vaccinated women in comparison with the men. Pregnancy can also substantially alter immune responses to vaccines. Concentrations of 17β -estradiol and progesterone are substantially higher during pregnancy than during other times in the female reproductive cycle. The polymorphism of genes encoding immunologically important proteins and also the level of steroid hormones in the vaccinated individuals (immune cells have receptors for hormones) may be responsible, among others, for the diverse response to the vaccine. Some researchers believe that a group of microorganisms, especially from the gastrointestinal tract and mucous membranes, should also be taken into account in the development of vaccines. Influence of microorganisms on the human immunity is significant, because they induce maturation of T lymphocytes function and affect the metabolism of drugs, toxins, and vaccines. It was shown that bacteria SFB (segmented filamentous bacteria) coordinate the response of Th1 helper and Th17 regulatory lymphocytes [20]. There was found an increased response to oral vaccines (rotaviruses, polio, *Salmonella*, *V. cholerae*) in individuals treated for 1–5 weeks with probiotics. Moreover, in neonates, 6-month administration of probiotics led to increased response to parenteral vaccines (anti-diphtheria, anti-tetanus HBV, against HBV). To date observations confirm the need for detailed and comprehensive study of microbiota composition and effectiveness of vaccines.

The aim of regenerative medicine is to use stem cells to treat damaged organs and tissues (P. Rieske, Medical University of Lodz). It is believed that whole organ transplantation in the future will be

increasingly replaced by stem cell transplantation. The most characteristic feature of stem cells is their ability to multiply and increasingly differentiate into organ-specific daughter cells that exhibit increased resistance to radiation and cytostatics. In recent years the concepts have appeared to use primitive pluripotent stem cells (PSC) which have the ability to differentiate into all cells of the body. PSC can be potentially extracted from embryos and from tissues (bone marrow). This type of stem cells isolated from adult tissues is called VSELs – very small embryonic-like stem cells. These cells are deposited in tissues during embryonic development as the population of PSC, which shows the expression of several markers of pluripotent cells (SSEA-4, Oct-4, Rex-1 and Rif-1). VSELs are mobilized into peripheral blood in case of acute damage to organs and tissues. At present, studies are being conducted on the use of VSEL stem cells and other in the treatment of non-specific enteritis (W. Marlicz, Medical University of Szczecin). It was found that the human and murine mesenchymal stem cells (MSC) administered parenterally reduce inflammation of the intestinal mucosa in models of *colitis* in mice. A particular challenge for clinical medicine is the prospect of regeneration of nerve tissue in degenerative diseases of central nervous system. Cell therapy aims is providing cellular structures that are the substitute for structures damaged by the disease process, and also stimulating immunoregulation which facilitates the action of corrective systems (multiple sclerosis). This therapy in Parkinson's disease will rely on the delivery of dopaminergic neurons from the „outside”. Clinical trials conducted to date bring about a moderate success. However, special hopes for future therapeutic use of stem cells are associated with such diseases as myocardial infarction, stroke, Parkinson's disease, diabetes, muscular dystrophies, toxic liver and kidney damage.

Microsporidia are obligate intracellular, primitive eukaryotes, lacking mitochondria and characterised by the typical structure of their spores. Intestinal microsporidiosis ranks among the most common causes of diarrhea in immunodeficient patients. Species identified as causing microsporidiosis in humans include the following: *Enterocytozoon bieneusi*, *E. intestinalis*, *E. cuniculi*, *E. hellem*. There were examined 92 children with congenital immunodeficiencies, and after liver transplantation, and 86 adults taking immuno-

suppressive drugs, and with chronic diarrhoea (M. Bednarska, A. Bajer, R. Welc-Falęciak, B. Samoliński, B. Wolska-Kuśnierz, I. Jankowska, T. Graczyk, E. Siński – Warsaw University, Medical University of Warsaw, Child Health Centre). In this study, faeces of patients were evaluated – smears stained with Chromotrp 2R technique, as well as by PCR method using primers specific for the SSU rRNA gene. There were found microsporidia *E. bienersi* and *E. intestinalis* in 13 tested subjects (7.3%). Intestinal microsporidiosis has been implicated as a major cause of chronic diarrhea in AIDS humans. The first time in Poland cases of microsporidiosis in HIV-infected patients were presented (M. Wesołowska, B. Knysz, M. Kváč, B. Sak, J. Janocha, D. Kvetonova, A. Gładysz – Medical Academy of Wrocław, Academy of Sciences of the Czech Republic). The coproscopic studies (chromotrope staining and fluorescence with Calcofluor White 2MR technique) and PCR method were performed and microsporidia were detected in 5 out of 50 tested patients; in 4 – *E. cuniculi*, and in 1 – *E. intestinalis*.

Babesiosis in humans is a potentially emerging tick-borne zoonotic disease caused by infection of red blood cells (RBCs) with various species of protozoan of the genus *Babesia* (Apicomplexa, Piroplasmida) (E. Siński, R. Welc-Falęciak, R. Poglód – Warsaw University and Institute of Hematology and Blood Transfusion, Warsaw). Babesiosis is transmitted primarily through the bite of an infected tick, typically *Ixodes* spp., although occasionally transmission occurs via transfusion of blood products collected from asymptomatic infected donors. Less common routes of transmission are transplacentally or perinatally (congenital babesiosis). In the literature, only three cases of congenital babesiosis are reported. For the period of 1979–2009, 159 *B. microti* transfusion-associated cases were documented in the United States. 46 patients in Poland with chronic Lyme disease, who had a history of confirmed contact with ticks, were examined [21]. In 14 of them (30.4%) *Babesia* spp. antibodies were found (IFA-indirect fluorescent-antibody technique) and in 1 person infection with *B. venatorum* was confirmed (molecular methods). In Poland, only seven human cases of babesiosis have been reported, including one imported from Brazil [22]. Babesiosis in humans particularly is the asymptomatic infection (when low parasitaemia lasts more than a year). The problem of human babesiosis in Poland and Europe

is still not fully appreciated, and the impact of this disease on transfusion medicine is undoubtedly underestimated. Transfusion-transmitted babesiosis might play a role in the emergence of this disease, especially in the northeast of Poland where there are well recognized endemic areas for *Babesia*.

Amoebiasis continues to be a significant worldwide health problem. It has been estimated that 500 million individuals are infected with *Entamoeba histolytica* and of these, 50 million would develop invasive disease. By contrast, *E. dispar* is regarded as a non-pathogenic commensal of man. Although biochemical, immunological, and genetic data account for a clear separation between *E. histolytica* and *E. dispar*, macroscopically both strains are undistinguishable. The diagnosis of *E. histolytica* infection has relied upon detection of the specific *E. histolytica* coproantigen (galactose-specific adhesin) in stool specimens, detection of antibodies to *E. histolytica* in patient sera or application PCR methods. In Poland, the great problem for diagnosis and treatment makes „imported” amoebiasis. Atypical clinical course of intestinal amoebiasis complicated by multiple liver abscesses in a patient after travelling to Cape Verde Islands was presented (M. Paul, J. Stefaniak – Medical University of Poznan). The authors discussed the diagnosis and treatment of 53-year-old patient. Before leaving, he had been consulted neither by tropical medicine specialist nor by travel medicine specialist, he did not undergo immunization recommended for international travellers, and did not respect the rules of tropical hygiene while staying abroad; during the trip there were no complaints. After returning to the country he developed fever up to 39°C preceded by chills, drenching sweats, muscle aches, general malaise, substantial weight loss, severe abdominal pain, loose bowel movement (3x/day with mucus). *E. histolytica* IgG was found by serological ELISA test, and computed tomography (CT) showed signs of a liver abscess. Additionally, indirect immunofluorescence assay (IFA) gave positive result.

Diagnostic and therapeutic difficulties of severe *P. falciparum* malaria based on own clinical experiences were presented (K. Mrówka, Medical University of Poznan). Severe human malaria is attributable to an excessive sequestration of *P. falciparum* – infected and uninfected erythrocytes in vital organs. This leads to obstruction of the microcirculation and results in dysfunction of multiple organs, typically the brain in cerebral malaria. The main clinical-pathological

features of severe malaria include central nervous system (CNS) involvement, severe anemia, renal failure, pulmonary dysfunction, shock, disseminated intravascular coagulation, hypoglycemia, metabolic acidosis and liver dysfunction. A case of severe malaria in 22-years-old man was presented. The patient stayed six weeks in Ghana where worked as missionary was presented. After return to Poland, he had following symptoms: up to 39°C fever, shivers, drenching sweat, general weakness, splenomegaly. Imported malaria is a major problem, which affects not only tourists but also missionaries, humanitarian volunteers, travellers to the tropics for professional purposes (businessmen, diplomats), immigrants (students, people visiting relatives in home countries) and soldiers of stabilization missions. In Poland mortality in malaria 7–16 times higher than in Western European countries [23]. Improper anti-malaria chemoprophylaxis and lack of pre-departure medical consultation with a tropical medicine specialist are important risk factors favouring the occurrence of infection with *Plasmodium* spp. in the travellers. Due to the indefinite symptoms of the disease, patients with malaria require professional and frequently multidisciplinary differential diagnostics.

Report on a severe case of symptomatic mansonellosis in an 11-year-old girl who took multiple trips to Egypt and the Dominican Republic was discussed (J. Stefaniak, M. Paul, K. Mrówka – Medical University of Poznan). Her last stay in Egypt was 2 months before, and in the Dominican Republic 22 months earlier. She stayed in high standard hotels but she was repeatedly bitten by insects. Before departure she had been consulted neither by tropical medicine specialist nor by travel medicine specialist, and had not been vaccinated in accordance with recommendations for international tourist traffic. She did not use anti-malaria chemoprophylaxis and did not follow tropical hygiene rules. There were the following symptoms in the patient: growing swelling of eyelids, inflammatory infiltration of soft tissue of eye socket, conjunctivitis, enlargement of lymph nodes. Immunochromatographic test for circulating filarial antigen (AD12) gave positive result and was confirmed by peripheral blood examination using Knott concentration method, showing single *Mansonella perstans* microfilaria in the afternoon hours (parasitaemia size: 0.5 microfilaria/ml). The authors emphasized the need for carrying out check-up to detect exotic diseases in tropical medicine

reference centres after returning from countries of different climate and sanitary zones. The most of illnesses contracted in the tropical and subtropical zones may be asymptomatic, or minimally symptomatic, or manifest clinically after a few months and even years after returning from the tropics.

Neurocysticercosis, which is caused by infection of the human with the cysticercus larva of *Taenia solium*, is the most common parasitic disease of the human nervous system. The recognition of neurocysticercosis is difficult, it require a multi-disciplinary approach of a parasitologist, a neurologist and a radiologist, while introduction of treatment in the early stages of invasion facilitates full patient recovery. The clinical manifestations depend on the number, and location of cysticercus cellulosae disrupting neural tissues. There are wide variations of clinical manifestations of neurocysticercosis (epilepsy, diplopia, vertigo). Three cases of central nervous system cysticercosis were presented among patients diagnosed in the Department and Clinic of Tropical and Parasitic Diseases in Poznan (Ł. Pielok, B. Skoryna – Medical University, Poznan). 32-years-old woman has been referred to the clinic with brain tumour suspicion. She had the following signs: right-sides paresis, aphasia and troubles with writing. Second patient – 63-years-old woman with neurological symptoms was hospitalised. She was previously diagnosed with multiple sclerosis. This woman had signs such as dizziness, equilibrium disturbances, diplopia and conjunctivitis. The third patient (32-years-old woman) showed epileptic seizures during night sleep.

Coprosopic study is still the reference method for detecting invasion of intestinal parasites. The most common causes of errors in the parasitological assessment of stool are non proper collection and storage of the material, lack of experience of the evaluator and inappropriate selection of the research method. Plant pollens are often misinterpreted as parasite eggs. Morphometric analysis of parasitic eggs from the human alimentary tract and plant pollens were demonstrated (K. Szwabe, P. Kurnatowski – Medical University of Lodz). The aim of the study was to demonstrate the differences and similarities between the morphological characteristics of selected intestinal helminth eggs and pollens of garden plants, commonly found in Lodz. Photographs of the compared objects, subjected to computerized image analysis using the

SCAN MULTI BASE programme, revealed in most cases a large morphological similarity of evaluated morphological parameters: length, width and length/width ratio of eggs with pollens similar to them. Small differences in the size of the evaluated eggs and pollens are not detected in routine microscopic evaluation. This may be the cause of diagnostic errors during testing for the presence of faecal eggs of intestinal parasites, and also in the helminthological evaluation of environmental samples.

The stool concentration procedures provide detection of developmental forms of intestine parasites. The literature data show that sedimentation methods are more sensitive than flotation. Using MINI PARASEP set in the diagnostics of intestinal parasitoses was presented (K. Szwabę, G. Lipowczan, P. Kurnatowski, J. Błaszowska – Medical University of Lodz). The efficacy of detection of intestinal parasites in 49 samples of faeces by traditional methods (direct formulation, the method of Kato-Miura, flotation by Faust) was compared with assessment by MINI PARASEP. Identical recognition – 19% of positive tests – was obtained evaluating the direct preparations, smears of Kato-Miura method and centrifuged sludges from the MINI PARASEP set. Parasites by flotation method were detected only in 8% of tested samples. Available on the market MINI PARASET concentrator based on sedimentation method can be successfully used for detection of developmental forms of intestinal protozoa and helminths in humans and animals. The advantages of above device are as follows: high density of the material, the possibility of semi-quantitative assessment of parasitic eggs in the sample, the minimum amount of reagents consumed, fast reading and ease of use. In addition, MINI PARASEP is a single use test, which eliminates the contamination of samples with environmental factors.

Antropozooses, which are difficult to diagnose and treat, pose still important epidemiological problem. There are human infections reported with parasites of domestic animals and livestock (*Toxocara canis* and *T. cati*, *Ascaris suum*, *Trichuris suis*, *T. vulpis* and *Capillaria* spp.). The growing number of newly registered cases of geohelminthosis, mainly toxocarosis, both in Poland and the world, calls for continuous monitoring of the sanitary status of the soil [24]. Preliminary assessment of the degree of soil contamination in

rural areas in the Lodz district with geohelminth eggs was demonstrated (P. Damięcka, P. Kurnatowski, J. Błaszowska – Medical University of Lodz). In this study, the authors have found high percentage (60 to 100%) positive samples of soil from agricultural fields fertilized with livestock manure. Out of the 285 isolated helminths as many as 250 eggs belonged to the genus *Ascaris*, 22 to *Toxocara*, and 10 eggs to *Trichuris*. 164 eggs were detected in samples from Kolumna, where the average density was 82 eggs/100g. The presence of parasitic eggs (mainly *Toxocara* spp. – 73.9%) was found while assessing soil samples from rural backyards, and the mean density of eggs ranged from 3 to 48 per 100 g of soil. The obtained results show a significant degree of soil contamination with geohelminth eggs in rural areas in the Lodz region. Special attention was drawn to the poor sanitary condition of backyards confirmed by a large density of *Toxocara* spp. eggs in the soil of the rural homestead areas.

Human dirofilariosis is a zoonotic filariosis caused by infection with several species of worms belonging to the genus *Dirofilaria*. The reservoir of these parasites are mainly dogs or cats, while the microfilaria vector are mosquitoes of the genus, i.a., *Culex*, *Anopheles*, *Aedes*. Although a man is an accidental host for *Dirofilaria*, these nematodes reach sexual maturity in a human body. There are two clinical forms of dirofilariosis in humans: pulmonary (mainly *Dirofilaria immitis*) and subcutaneous (*D. tenuis* and *D. repens*). In Europe, the endemic area of *D. repens* occurrence contain primarily Mediterranean countries as well as Hungary, Ukraine, Slovakia and the Czech Republic. The diagnostic methods used for differentiation of nematodes of *Dirofilaria* spp. isolated from humans were presented (A. Masny, H. Żarnowska-Prymek, D. Cielecka, R. Salamatin, E. Dove – National Institute of Public Health-National Institute of Hygiene, Warsaw). Determination of parasite species is based on evaluation of stained histological preparations from subcutaneous tissue nodules which contain sections of the nematode. The most important morphological characteristics of the adult form of the parasite are taken into consideration (the maximum body diameter, thickness and striation of multilayer cuticle, the presence of a double uterus, arrangement of fibres and muscle cells, hypoderma appearance and its lateral ridges). The assessment of morphological microfilaria detected in the blood of

the host is often insufficient for the determination of the species. In such cases, membership of a species should be confirmed by molecular methods. In the PCR techniques species-specific markers are used (mitochondrial and ribosomal) and ITS universal markers (spacer region of rDNA).

Most infants with congenital *Toxoplasma gondii* infection have no symptoms at birth, but many will have retinal disease or neurologic abnormalities later in life. Early detection and treatment of congenital toxoplasmosis may reduce these sequelae. Congenital toxoplasmosis is treated with pyrimethamine and sulfadiazine in combination therapy for a period of 2–6 months. The next step in the therapy is administration of Fansidar until the end of the first year of age. Pyrimethamine is an anti-protozoal agent irreversibly blocking the synthesis of nucleic acids by inhibiting dihydrofolic acid reductase. Therapeutic range of this drug is 0.08–0.6 µg/ml of serum. The report on monitoring, in clinical practice, of plasma pyrimethamine concentration in infants with congenital *Toxoplasma gondii* infection was presented (B. Lipka, B. Milewska-Bobula, M. Filipek – Child Health Center, Warsaw). In this study, pyrimethamine levels were monitored in the plasma of 24 infants aged 1–5 months with congenital toxoplasmosis. The high performance liquid chromatography (HPLC) was used to identify pyrimethamine concentration. It ranged between 0.01–1.2 µg/ml. In 14 infants the drug concentration was within the therapeutic range, in 7 patients it was significantly too low, and in 3 the concentration above the therapeutic range was reported. The obtained data suggest that monitoring of pyrimethamine in plasma can improve the safety and efficiency of treatment and enable selection of appropriate, individual dose. The most common side effect of pyrimethamine occurring even when using the recommended dose is neutropenia. That's why it is necessary to check the blood count regularly during treatment.

During the development and effector phases of the anti-*Toxoplasma* response, the immunological system of a host is involved in several complex interactions with the endocrine system, and prolactin (PRL) is one of the most important hormones involved in immunoregulation. The protective effect of prolactin (PRL) in *T. gondii* infection was demonstrated in experimental models of toxoplasmosis in mice [25]. Moreover, the prevalence of *T. gondii* invasion in humans was determined depending on the level of PRL.

Published results of numerous researchers suggest that the state of hyperprolactinaemia may inhibit the development of invasions caused by *T. gondii* [26]. But there is still unanswered questions: whether the protective effect of PRL is due to the direct influence of the hormone on extracellular forms of *T. gondii*, or is the result of the immunoregulatory role of PRL which, while activating the host cells, leads to inhibition of parasite replication and, consequently, to its gradual eradication. The literature data show that PRL activates intracellular killing of protozoa and releasing of IL-1β, IL-3 and IL-6, what results in the decrease in the number of cysts formed in the brain of experimental animals. The report on the influence of recombinant and serum prolactin on *T. gondii* growth in human peripheral blood mononuclear cells (K. Dzitko, H. Ławnicka J. Gatkowska, B. Dziadek, J. Komorowski, H. Długońska – Lodz University, Medical University of Lodz) was presented. The authors of this study evaluated the effect of this hormone on the rate of proliferation of *T. gondii* in peripheral blood mononuclear cells (PBMCs). There was observed a significant decrease in intracellular multiplication of parasites in the presence of high concentrations (91.00–175.00 ng/ml) in both the serum prolactin (SPRL: 60.04 ± 16.09%), and the recombinant (rhPRL: 78.69 ± 14.40%), what confirms that the state of hyperprolactinemia may inhibit the growth of *T. gondii* in host cells.

Mycological topics of this congress included characteristics of different clinical forms of fungal infections (central nervous system, oral cavity and pharynx, paranasal sinuses, skin and nails), still current problem of antimicrobial susceptibility of fungal strains, diagnostic and therapeutic difficulties in zoonotic mycoses.

The risk factors, clinical symptoms, microscopic image of CNS mycosis (aspergillosis, mucormycosis, candidiosis, coccidioidomycosis and cryptococcosis) were discussed (B. Sikorska, Medical University of Lodz). Moreover, rare clinical cases of central nervous system mycoses leading to coma and death of patients were presented. The CNS mycoses most commonly occur in people with compromised immune systems (AIDS, immunosuppressive therapy), or chronically ill (diabetes, cancer), and are manifested by symptoms of meningitis, abscess formation or granulomous changes, infarctions and micro-infarcts in the brain. *Cryptococcus neoformans*, *Candida albicans*, *Coccidioides immitis* are common causes of fungal meningitis. Fungi of the

genus *Aspergillus* and *Mucor* are known to cause mass lesions in brain. The morphology and size of the organism determine the pathology of CNS lesions. Small yeast forms (*Coccidioides*, *Cryptococcus*, *Candida*) reach the small arterioles and capillaries producing meningitis and ischemic lesions. Intermediate-sized pseudohyphae (*Candida*) occludes small vessels in the microcirculation to produce local areas of tissue necrosis that evolve to abscess formation. The large hyphal forms (*Aspergillus* spp., *Mucor* spp.) of variable size obstruct large and intermediate-size arteries and occasionally veins, giving rise to large infarcts.

The results of long-standing mycological analyses of biological materials from selected organ ontocenoses were reported (M. Dynowska, K. Góralska, P. Troska, G. Barańska, A. Biedunkiewicz, E. Ejdyś, E. Sucharzewska – University of Warmia and Mazury, Hospital of the Ministry of Internal Affairs and Administration, Oncology Centre in Olsztyn, District Hospital, Biskupiec). Changes in the number of taxa of fungi isolated from the respiratory and digestive tract of patients from the Hospital of the Ministry of Internal Affairs and Administration, Oncology Centre in Olsztyn and the District Hospital in Biskupiec in the years 1990–2010 were noted. Recording up to 9 taxa more in the respiratory tract than in the alimentary tract confirms earlier assumptions that fungi in the respiratory system find better conditions for survival and development (greater stability, oxygen supply) than in gastrointestinal tract, what does not undermine the fact that the gastrointestinal tract is the most common way of intra-systemic fungal transmission. During above period, *Paracoccidioides brasiliensis*, *Candida lactis-condensi*, *Oosporidium margaritifera*, *Rhodosporidium diobovatum* and *Rhodosporidium kratochvilovae* were isolated in Poland for the first time. Attention was drawn to the gradual expansion of the taxonomic and ecological spectrum of fungi in human organ ontocenoses and increasingly noted multifocal settlements of fungi, which greatly raises the risk of the spread of fungal invasion via blood to different organs. The number of persons with compromised immune systems and increased sensitivity to fungi is growing, and also more iatrogenic factors appear enabling the growth of expansiveness of fungal saprotrophs that reveal accelerating pathogenic potential in man, treating ontosphere as one of the natural reservoirs.

Depending on the nature of the fungal infection,

topical or systemic antifungal drugs may be used. Despite extensive research dedicated to the development of new therapeutic strategies, there are only a limited number of available drugs to fight against invasive fungal infections. The new possibilities for treatment of mycoses of oral cavity and pharynx were delivered (P. Kurnatowski, Medical University of Lodz). The increasing incidence of invasive fungal infections is the result of many factors, including an increasing number of patients with severe immunosuppression. The high risk group for the incidence of mycoses, among others, include: the persons with neutropenia below $0.5 \times 10^9/l$ persisting over 5 weeks, subject to corticosteroid therapy ($2 \text{ mg/kg} > 2$ weeks), showing signs of transplant rejection, and colonization by *C. tropicalis*. Despite improvement of antifungal therapies over the last 30 years, the phenomenon of antifungal resistance is still of major concern in clinical practice. Currently, it is noted a decrease in infections caused by *C. albicans* from 90 to 30% and an increase in prevalence of strains resistant to antifungal drugs: *C. krusei*, *C. tropicalis* (from 2 to 24%), *C. glabrata* (from 2 to 26%), *C. parapsilosis* (from 10 to 20%). In addition, there has been an increase in the frequency of infections with drug-resistant strains of *Fusarium solani*, *Mucor* sp., *Rhizopus* sp., *Cryptococcus neoformans*, *Aspergillus* sp. It is worth noting that fungemia in approximately 30% of cases results in death of the patient. One of the antifungal drugs with a broad spectrum of action is Noxafil (posaconazole) which can be used to treat mycoses caused by *Candida albicans*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, *C. neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Fusarium solani*, *Scedosporium apiospermum*, *Rhizopus* sp., *Mucor* sp., *Rhizomucor* sp. Indications for the drug use include candidiasis of the oral cavity and pharynx (first-line therapy), aspergillosis, fusariosis, chromoblastomycosis, coccidioidomycosis. Noxafil is effective on fungal strains resistant to azoles or amphotericin B. It is recommended in patients with severe mycosis or reduced resistance, in whom poor response to topical therapy is expected. It is also indicated for the prevention of invasive mycoses in patients receiving chemotherapy (acute myeloid leukaemia, myelodysplastic syndrome), and bone marrow transplant.

Pathogenic fungi play a significant role in

chronic rhinosinusitis. In about half of the patients not only *Candida* species are detected, but also *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. In the last decade increased prevalence of fungal invasions within the nasal cavity and paranasal sinuses has been recorded. Several factors favour expansion of fungal sinusitis, including, i.a., frequent use of antibiotics that disturb the physiological bacterial flora, or increasing number of immunocompromised patients. A particularly important factor facilitating the current development and spread of fungi is contaminated environment. Also advances in diagnostic testing (mycological, serological, histopathological and radiological) allow for identification of fungal etiological factors. In patients undergoing surgery for chronic rhinosinusitis in the Department of Otolaryngology and Laryngological Oncology, most frequently isolated from the mucous membrane fragments of the ethmoidal bulla region were fungal strains of the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Rhizopus*, *Oidium* and *Rhodotorula* (M. Twarużek, J. Grajewski, P. Winiarski, E. Soszczyńska – Kazimierz Wielki University, Bydgoszcz).

Identification of fungi from the genus *Penicillium* originating from clinical specimens in the majority of laboratories is based on culturing over Sabouraud medium with chloramphenicol in 37°C, then micro- and macroscopic evaluation. The growth of strains of *Penicillium nalgoviense* (from BAL), *P. ochrochloron* (from bio-aerosol of laboratory area) and *P. simplicissimum* (from nasal cavity) on different substrates and at different temperatures was compared (E. Ejdys, M. Dynowska, M. Roslan, K. Góralaska, A. Biedunkiewicz, E. Sucharzewska – University of Warmia and Mazury, Independent Public Centre of Tuberculosis and Lung Diseases, Olsztyn). According to the authors, the clinical material from the respiratory tract should be incubated in parallel at least at two temperatures: 37°C (testing the potential pathogenicity of *Penicillium* isolate) and 25°C (quicker development of morphological features needed to identify the fungus). Although the fungi of *Penicillium* genus generally grow better on Sabouraud agar without antibiotics, it is advisable to conduct observation also on the Czapek-Dox medium. Differences in morphology of colony observed on both the media are helpful in efficient identification of the *Penicillium* genus.

Fungi strains of *Fusarium* species are common

soil phytopathogens, easily invading weakened organisms. The fungi, forming hyaline hyphae, may be the cause of mycoses named hyalohyphomycosis. Literature describes rare cases of isolation of *Fusarium oxysporum* and/or *F. solani* from necrotic changes in skin and subcutaneous tissue, cornea, surgical wounds, and the inter-digital skin lesions and nails in surgical, dialysed and immunocompromised patients. The rare detection of *Fusarium* fungi may be the result of errors in the standard diagnostics – caused by the action of actidion added to the standard Sabouraud media as an inhibitor of mould growth. Analysis of several cases of hyalohyphomycosis caused by *F. solani* and *F. oxysporum* recorded in Olsztyn and Lidzbark Warminski showed that the PDA medium (potato-dextrose agar) should be introduced into routine diagnostics of fungi isolated from skin lesion and surgical wounds (K. Góralaska, M. Dynowska, G. Barańska, P. Troska, M. Tenderenda – University of Warmia and Mazury, Hospital of the Ministry of Internal Affairs and Administration, Oncology Centre in Olsztyn, District Hospital, Biskupiec, Independent Public Centre of Tuberculosis and Lung Diseases, Olsztyn).

Madura foot (lat. *mycetoma*) is a chronic infectious disease of the skin and subcutaneous tissue characterized by progressive course which may affect muscles, bones and neighbouring organs. The name derives from the region of Madura in Madras (India), where it was first described in the nineteenth century and it is now observed endemically in Africa, Latin America, India, Pakistan and Australia. The etiological factor may be different microorganisms – actinomycetes (*Nocardia asteroides*, *Nocardia madurae*, *Actinomadura madurae*, *Actinomadura pelletieri*, *Streptomyces somaliensis*, *Nocardia brasiliensis*, *Nocardia caviae*) or numerous pathogenic fungi (*Madurella mycetomatis*, *Madurella grisea*, *Pyrenochaeta* spp., *Scedosporium apiospermum*, *Fusarium* spp., *Acremonium* spp., *Aspergillus nidulans*, *Neotestudina rosati*, *Pseudoallescheria boydii*, *Exophiala* spp., *Leptosphaeria senegaliensis*). An exceptional case of bilateral maduromycosis in 51-year-old woman after a 3-week stay in Ethiopia was presented (M. Paul, Ł. Pielok, J. Stefaniak – Medical University, Poznan). The patient was admitted to the Department of Tropical and Parasitic Diseases, Medical University in Poznan with suspicion of malaria. The clinical picture was manifested by 39°C fever, chills, substantial general weakness, muscle aches,

headaches, feeling cold. Moreover, bilaterally on both feet numerous painful inflammatory lesions in the form of deep skin ulcers with purulent content were observed, accompanied by swelling of inflamed subcutaneous tissue. *Staphylococcus aureus* and *Streptococcus pyogenes* were grown in bacteriological cultures from swabs of the patient's feet ulcers, and also, the direct preparation from purulent content revealed *Actinomyces* sp. Combined chemotherapy (Co-trimoxazole, amoxicillin and clavulanate, ketoconazole, clindamycin, ciprofloxacin) for a total period of 2 months was introduced, which brought significant alleviation of maduromycosis symptoms.

The predominant form of chronic dermatophytoses are athlete's foot (*tinea pedum*) and feet onychomycoses (*tinea unguium*) caused mainly by *Trichophyton rubrum*. The two types of mycoses are considered the civilization-related diseases. European evaluation *Achilles* revealed the prevalence of athlete's foot of 22% and feet onychomycoses – 23%. Between sportsmen it raised to 31% (runners) or 63,6% (swimmers). Also work environment may favor the fungi invasion. There are different types of onychomycosis: Distal Lateral Subungual Onychomycosis DLSO, Proximal Subungual Onychomycosis PSO, White Superficial Onychomycosis WSO, Total Dystrophic Onychomycosis TDO and Endonyx Onychomycosis EO. The genotype typing of the fungus strains isolated from patients may be useful in epidemiological studies. The differentiation of *T. rubrum* genotype based on the analysis of polymorphism in regions of TRS-1 and TRS-2 showed seven patterns, and in RAPD reactions (Baeza primers 1 and 6) only two pairs of strains presented the same genotype, the others presented unique genotypic patterns (A. Hryncewicz-Gwóźdź, T. Jagielski, A. Sadekierska-Chudy, K. Kalinowska, K. Pawlik – Medical Academy, Wrocław). This indicates significant genetic variety of tested *T. rubrum* population. However, analysis of fluconazole and itraconazole sensitivity of strains qualified for different genotypes did not show a strict correlation between genetic types and their susceptibility.

The other dermatophyte *Trichophyton verrucosum* is a zoophilic species, most often causing dermatomycoses in cattle. The incidence of the fungus infections in humans is small (<1% of all skin fungal infections), it causes deep lesions of *kerion Celsi* type. Humans are mainly infected through direct contact (farmers, cattle breeders),

occasionally by human to human transmission. Mycosis is often observed in children and young adults; changes are visible on head, beard or other not protected body parts. In cattle infected hair under Wood's lamp show fluorescence, which is not observed in humans. Zoonotic *tinea barbae* may show course not only with deep skin changes, but also with intensive pain (Z. Adamski, A. Grajewska, A. Kubisiak-Michalska, H. Kubisiak-Rzecznyk – Medical University, Poznan). Dermatophytes are invasive fungi and/or allergizing agents – in skin, subcutaneous tissue and in vascular system mycides (dermatophytids) may develop, as response to fungus antigens within 4–6 weeks of invasion (e.g. erythema nodosum picture with reactive arthritis). Systemic treatment with antifungals and a non-steroidal anti-inflammatory drug are reducing symptoms of the organism reaction.

A zoophilic species *Trichophyton equinum* can spread from the environment of infected animals (horses, dogs) to healthy ones, with the incidence of invasion reaching up to about 20%, while in humans is rare. In the analysis of 1195 mycoses in Poland (1984–1995), the presence of *T. equinum* was not detected in any of the cases (A. Grajewska, Z. Adamski, H. Kubisiak-Rzecznyk, A. Banaszak – Medical University, Poznan). The fungus species case of *tinea capitis* in 4-year-old boy was probably caused by a horse, on which the boy rode in kindergarten. Griseofulvin therapy was introduced for a period of 13 weeks yielding satisfactory clinical outcome without observed side effects. This drug is effective and safe in the treatment of *T. equinum* infection, possible for use in children.

Fungi of the genus *Malassezia* causing pityriasis versicolor (*tinea versicolor*) belong to dimorphic fungi, which use lipids as a carbon source. Fats present in human skin sufficiently secure their demand for fatty acids. 12 lipodependent species (*M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, *M. slooffiae*, *M. dermatis*, *M. japonica*, *M. nana*, *M. yamatoensis*, *M. equina*, *M. caprae*) and 1 non-lipodependent (*M. pachydermatis*) – causing skin lesions in animals have been detected so far. The natural substance that is produced by *Malassezia* – saturated dicarboxylic azelaic acid – competitive inhibits tyrosinase, a key enzyme in human melanogenesis. This substance contributes to the formation of hypopigmented skin spots [27]. Analysis the patients' data showed the greatest incidence of *tinea versicolor* in young people in relation to other age groups (J. Kwaśniewska, E.

Morus-Urbańska, A. Jaskółowska – Medical University of Lodz). The most frequent location of clinical changes concerned the skin on the back (significantly more frequent in women than in men) or both back and chest. The only favourable factor of infection was seborrhea, predominantly found in patients from the youngest age group.

Water ecosystems and animals closely connected with them are rich reservoirs of factors potentially pathogenic for humans. Special role belong to water and mud birds – cormorants and Charadriiformes. Evaluation of alimentary tract (beak, intestine and cloaca) and respiratory system of these birds revealed 14 species of 8 genera of yeast-like fungi and one species of mould fungus (A. Biedunkiewicz, J. Dziekońska-Rynko, M. Dynowska, E. Sucharzewska, E. Ejdys, K. Góralska – University of Warmia and Mazury, Olsztyn). *Candida krusei* was dominating species (28% of positive isolates). In all tested birds the presence of larvae and adult nematodes *Contracaecum rudolphii* was found. Man can be a paratenic host for this cosmopolitan parasite. Also, all the birds were infected with *Paradilepis scolecina* tapeworm which does not invade humans. It is worth pointed out, that the human body may be a new habitat for fungi and parasites, for which the reservoir are wild birds. These vertebrates are still in contact with water reservoirs and easily translocate in man' surrounding environment.

Pneumocystis jirovecii is the causative agent of *Pneumocystis* pneumonia (PCP), one of the severe infections in premature infants, dystrophic children and children with immunological disfunctions or highly hypoalimmented. Serologic studies have shown that children are exposed to *P. jirovecii* early in life. It may be transplacental or airborne acquisition of the disease from human sources. Evaluation of group of children (age 7 days to 24 months) hospitalized with acute respiratory infections revealed *P. jirovecii* prevalence of 16% [28]. From the group of 70 children, aged from 11 days to 5 years, treated in Warsaw hospitals with acute respiratory infections, the aetiology of which was defined as viral basing on clinical symptoms, nasopharynx swabs were collected (E. Gołąb, W. Rozej-Bielicka, K. Pancer – NIPH-NIH, Warsaw). In 86% of patients the infection with at least one of RNA viruses was detected: RSV, hMPV, PIV 1-3, enteroviruses. The use of nested PCR method allowed for *P. jirovecii* infection identification in two (2.9%) of the examined

children. In one of them co-infection with parainfluenza virus type 3 was detected. The authors indicated that the primary fungus infection may manifest as acute airways inflammations suggestive of viral infections. In the differential diagnosis of *Pneumocystis* infections in children molecular methods are useful for their high sensitivity of samples testing of material collected noninvasively.

Deposits of cells, forming complex surface-associated communities enclosed in a matrix produced by them are named biofilms. Biofilm-associated microorganisms have been shown to be the cause of several human diseases, such as native valve endocarditis and cystic fibrosis, and to colonize a wide variety of medical devices. The study of biofilm formation in YNB and serum by *Candida albicans* strains isolated from fungal infections of the mucous membranes of the oral cavity, vagina and gastrointestinal tract was conducted in flat bottom titration plates (P. Krzyściak, Jagiellonian University Medical College, Cracow). To quantitative assessment of biofilm formation the light microscopy and evaluation with crystal violet staining were used. There were no differences in the ability of biofilm formation among examined 41 strains of *C. albicans* from different ontocenoses in both substrates. Serum, however, increases several times the ability to form biofilms by these strains.

Molecular analysis of fungi strains allows understanding the genetic diversity and structure of fungus populations. Strains of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum* derived from humans and animals from Lodz and Lublin were examined with application of the RAPD method based on (GACA)₄ and (ACA)₅ primers (J. Dębska, A. Ciesielska, A. Jaworski – University of Lodz). The study revealed differentiation of studied strains within species *T. mentagrophytes* and *T. ajelloi* (both in not numerous number of strains) and lack of differentiation of 59 strains of *M. canis*. The authors emphasized that in order to confirm the obtained preliminary results further research is needed on a larger collection of strains and based on other molecular methods (PCR-MP).

Scopulariopsis brevicaulis causing mainly superficial infections (nail fungal infections, less often skin) may also be the cause of invasive mycoses (i.a., *keratitis*, *endophthalmitis*, *endocarditis*, *sinusitis*, pulmonary aspergillosis, cerebral mycosis). Literature data indicate that this species is resistant to Amphotericin B, 5-Fluorocytosine, Terbinafine,

and azole drugs. Assessment of antimicrobial susceptibility carry out *in vitro* using agar diffusion method revealed sensitivity of 31 clinical and 4 references *S. brevicaulis* strains to 12 antifungal drugs (M. Skóra, A.B. Macura – Jagiellonian University Medical College, Cracow). Antifungal activity against all tested strains was demonstrated for terbinafine, ciclopirox and clotrimazole, but in the case of the latter drug 87% of strains were weakly sensitive. Multiple drug resistance of *S. brevicaulis* may require combination therapy, and/or surgical procedures. The authors pointed out that promising result have been obtained by a team of Cuenca-Estrella, who have demonstrated that certain combinations of antifungal drugs (Posokonazole plus Terbinafine, Voriconazole plus Terbinafine, Itrakonazole plus Terbinafine, Amphotericin B plus Caspofungin, Posokonazole plus Caspofungin, Voriconazole plus Caspofungin) exhibit synergistic activity *in vitro* against *S. brevicaulis* [29].

The health implications of the fungal contamination of indoor air are still an issue of increasing concern in recent years. It is recognized that fungi can cause spectrum of illnesses in humans, ranging from rhinitis to invasive diseases. Especially important is the role of environmental fungi in causing opportunistic infection in immunocompromised patients in hospitals. Contamination of air and walls in one of Cracow hospitals, in five operating rooms equipped with air conditioning with absolute filters was evaluated for 5 days (twice a day, 7.00 am and 7.00 pm) (A. Gniadek, A. B. Macura – Jagiellonian University Medical College, Cracow). Air samples were taken using a MAS 100 (Merck) device, and imprints from the walls were collected by Count-Tact method. Of the 50 air samples in 48 cases the fungal growth was observed (mean numbers 5–100 cfu/m³). Only three samples exceeded the permissible concentration of microorganisms for highly aseptic operating rooms according to the Polish and Swiss requirements. The dominant fungi isolated from the operating theatres environment were microorganisms from species *Aspergillus* (35%), *Penicillium* (24%) and *Cladosporium* (19%).

Bioaerosol consisting of viruses, bacteria, moulds and yeast fungi, Acaridae – stored product mites and plants pollen may constitute threat to human health. Fungal spores depend on diameter, can be deposited in some parts of respiratory tract (*Alternaria alternata*: diameter > 10 µm – upper

airways (nose and throat); *Cladosporium macroparvum*: diameter 5–10 µm – upper and lower airways; *Aspergillus fumigatus*: diameter 2–5 µm – lower airways) and cause allergies or dysfunctions of the tract. The analysis of seasonal biodiversity of pathogenic fungi present in the air of poultry house and around farm by the collision method (aeroscope MAS 100 Merck) revealed, out of 90 air samples, 26 fungi species of the genera: *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Exophiala*, *Fusarium*, *Chaetomium*, *Scopulariopsis*, *Acremonium*, *Candida* and *Rhodotorula* (K. Plewa, E. Lonc – Wrocław University). The greatest biodiversity of fungi was in the spring/summer, and the highest concentration of fungi in the autumn. Outdoor air was characterized by greater biodiversity and lesser concentration in comparison with the interior of the poultry houses, where the cfu/m³ values exceeded the recommended level.

Fungi and fungus-like organisms, together with some bacteria species, play significant role in the aquatic ecosystems – take part in decomposition of organic matter commonly found in inland waters. But also, fungi potentially pathogenic and allergenic for humans may be present in water reservoirs. For evaluation of the organisms occurring in the Horodnianka river water, near waste landfills in the vicinity of Białystok, the method of plant and animal baiting was used (B. Kiziewicz, E. Zdrojkowska, B. Gajo, A. Godlewska, E. Muszyńska, B. Mazalska – Medical University of Białystok). 26 species of fungi and fungus-like organisms were cultivated, including potentially pathogenic, of the genera: *Alternaria*, *Aspergillus*, *Fusarium*, *Lagenidium* and *Penicillium*. The presence of sewage fungus *Leptomitium lacteus* was also detected which, in addition to bacteria *Sphaerotilus natans*, *Beagiatoa alba* and *Zooglea ramigera* may be a good biological indicator of α -mesosaprobic zone.

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