 

Review articles

# Parasitoses and mycoses – still current public health hazards[1]

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**ABSTRACT.** Current environmental aspects of parasitological and mycological diseases, diagnostics problems and some mechanisms of pathogens' action facilitating invasion of human organisms have been presented. Imported humans parasitoses, difficulties in diagnosis and treatment, possibility of expansion of the endemic pathogens' occurrence ranges have been discussed. Mycological topics included evaluation of the role of birds as vectors of potentially pathogenic fungi in biosphere, threats connected with biofilms formation in hospital and home environments, and interrelations among microorganisms in such biofilms.

**Key words**: medical parasitology, mycology, parasitoses, mycoses

Despite advances in biological and medical sciences, fungi and parasites still may cause considerable health problems for contemporary humans. Knowledge concerning pathogenic fungi, protozoans, helminths and arthropods is rising; new methods of diagnostics and prevention are introduced, but still expanding of the occurrence ranges of some of these pathogens is observed. A number of factors may be the cause i.a., supplying of food and drinking water of inadequate quality, poor level of hygiene, also in health care units, inappropriate management of human and animal excreta and intensity of human migration, as well as changing of climatic zones while travelling. In addition, pathogens and potentially pathogenic organisms are changing some of their features, which facilitates invasion to human organism, survival and development in or over the host. Some of the mentioned problems were presented during 52nd Clinical Day of Medical Parasitology (Lodz, 17th May 2013).

Mechanisms of adaptive variation, their role for the survival of organisms in unfavorable conditions, for the evolution of living organisms on Earth, are still under discussion. Neo-Darwinism and quasi-Lamarckism are now recognized as equivalent theories describing various aspects of the interaction between populations and the environment. One of fundamental factors of evolution is mutation conditioning variation. The new hereditary modification is the result of accidental and random genetic changes (spontaneous mutations), but also new genetic changes, called adaptive mutations, may be stimulated by stress, and may allow for adaptation to the stress. Adaptive mutations have been defined as a group of spontaneous mutations that occur in populations of bacteria usually after 3–7 days of incubation on selective growth media, particularly under conditions of very strong inhibition or reduction of DNA replication and cell division. Such mutations are an example of a strategy to adapt to environmental conditions. Possibilities of adaptive rearrangements of bacterial genomes in response to environmental stresses have been presented by the team from Lodz (A. Jaworski, A. Ciesielska, M. Gadzalski – University of Lodz). They discussed SOS-induced mutagenesis, stimulation of natural genetic engineering systems of bacteria and other adaptive systems.

Under the conditions of environmental stress, the mechanisms and processes responsible for HGT (horizontal gene transfer) are induced or amplified.

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While HGT is common among prokaryotes, it is rarely observed among eukaryotes. However, phylogenetic analyses of the occurrence of the gene encoding the fructose transporter (FSY1) in fungi *Ascomycota* indicate that interspecies HGT could also have a significant impact on shaping the genomes of fungi [1].

Pathogenicity of microorganisms depends on many pathogenic determinants of strains. One of these is the ability of a microorganism to form a biofilm, which results in significant clinical implications such as multiple increase in drug resistance and the ability of microorganisms inhabiting the internal structure of the biofilm to avoid the host immune response. Intensive studies *in vitro* and *in vivo* of single and mixed species biofilms have been carried out recently. It has been shown that a specific chemical communication system called quorum sensing functions between the microorganisms belonging to one or different species [2]. Several synergistic and antagonistic interactions between some species of bacteria and fungi have been described. It has been found that *Pseudomonas aeruginosa* bacteria inhibit the growth of *Candida albicans* pseudohyphae. On the other hand, *C. albicans* blastospores, owing to produced farnesol, affect the production of signaling molecules in *P. aeruginosa* [3]. The streptococci *Streptococcus gordonii*, which form natural microbiota of the oral cavity, can stimulate the growth of *C. albicans* and biofilm formation in this ecological niche. However, the multiplication of *C. albicans* in the vagina is inhibited by the population of lactic acid bacteria *Lactobacillus* spp. that produce hydrogen peroxide and various types of bacteriocins [4]. The influence of co-incubation of bacterial species from the genus *Lactobacillus* with cells of *C. albicans* on the formation of single species (fungus) and mixed (bacteria-fungus) biofilms has been investigated (P. Krzyściak, A. Buda, G. Gołąb – JU-CM, Cracow). It was shown that prior incubation of lactic acid bacteria (*L. acidofilus, L. fermentum, L. rhamnosum*) with *C. albicans* blastospores did not affect the quality of the fungal biofilm created after co-culture. The presence of *C. albicans* in mixed biofilm favored the survival of *L. fermentum* and *L. rhamnosum*, and completely inhibited the growth of *L. acidofilus*. Moreover, while studying the interactions between *P. aeruginosa* and *C. albicans*, previously detected by other researchers antagonistic interactions between these species were confirmed [3].

One of the determinants of fungal strain pathogenicity is the ability to secrete proteolytic enzymes. Aspartyl proteases (Saps), encoded by a family of 10 SAP genes, are probably responsible to a large extent for the pathogenicity of *C. albicans* [5]. This is shown by results of studies on SAP genes expression and experiments using inhibitors of SAP or SAP knockout mutants. *In vivo* analysis of SAP genes expression profile in *C. albicans* isolates obtained from the swabs of the oral mucosa and vagina confirmed different expression of the genes, which correlated with the clinical form of candidosis and anatomical location of the obtained strains [6]. It was further disclosed that different strains of *Candida* genus have a different number of genes encoding aspartyl proteases Saps. So far 9 SAP genes have been detected in strains of *C. dubliniensis*, 4 in *C. tropicalis* and 2 genes in the strains of *C. parapsilosis*. In a preliminary study, a team from Lodz (K. Kuba, P. Kurnatowski, B. Modrzewska – Medical University of Lodz) compared the frequency of detected SAP1-10 genes in fungal strains of the genus *Candida* isolated from dental and oncological patients, and people without changes in the oral cavity (control group). In the strains: *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. humicola* at least one gene from group SAP1, SAP2 or SAP3 was detected. Most *C. albicans* isolates possessed aspartyl protease genes SAP1-3; the highest proportion of such strains was isolated from dental patients. Currently, research teams are looking for new and effective methods to assess SAP genes expression. As can be seen from the literature, for this purpose the real-time RT-PCR assay can be successfully used which allows to quantify the level of an mRNA transcript of each SAP1-10 gene present in *C. albicans* isolates from patients in various clinical conditions [7].

Intra- and inter-human transmission of fungal strains and growing drug resistance are current therapeutic problems [8]. The occurrence of symptoms and the course of mycosis depends primarily on the general and immunological condition of the patient and pathogenicity of strains. Successful treatment of fungal infections is primarily determined by proper clinical and mycological diagnosis. The differences in morphological, adhesive and biochemical properties among the strains responsible for asymptomatic and symptomatic invasion have been demonstrated. Hence the need to know the role of fungi in the pathogenesis of human diseases, and also biotyping

of strains isolated from patients, determining their pathogenicity and antimicrobial susceptibility. The properties of various *Candida* species from patients treated in ambulatory and hospitalized were compared (B. Modrzewska, P. Kurnatowski – Medical University of Lodz). Adhesive, biochemical properties and enzymatic activity of 134 fungal strains isolated from the oral cavity of dental patients, oncological patients and students of Medical University of Lodz without changes in the oral cavity (control group) were determined. Strains belonging to all the groups were characterized by producing a large variety of hydrolytic enzymes, with the strongest activity of leucine arylamidase (98.5%) and esterase (97.8%). The highest hydrolytic properties showed the strains derived from cancer patients. The lowest activity of hydrolytic enzymes, the smallest variety of biotypes and adherence properties were recorded among strains isolated from patients without clinical symptoms.

In the course of other experiments the prevalence of fungi isolated from patients with colorectal cancer was evaluated (P. Troska – UWM, Warmia and Masuria Oncology Centre, Olsztyn). 188 isolates from 92 patients possessed yeast-like fungi and yeasts belonging to 21 species from eight genera: *Candida, Debaryomyces, Dipodascus, Geotrichum, Saccharomycopsis, Saccharomyces, Trichosporon,* and *Rhodotorula*. After cancer therapy a significant increase in the prevalence of fungi was reported. In addition, fungi occurred in all tested ontocenoses in most patients. The results confirm the need for mandatory mycological testing in patients with colorectal cancer and suggest introduction of anti-fungal prophylaxis alongside the anti-cancer therapy.

The introduction of modern methods of invasive treatment and broad use of antimicrobial, immunosuppressive and hormone therapies promote the development of iatrogenic mycoses. The spectrum of fungal species that cause infection in humans is constantly changing [9]. The *Candida albicans* species dominated (>90%) until the introduction of azole treatment. There is currently an increase in cases of candidosis caused by species other than *C. albicans*, which account for about 40% of the strains of fungi isolated from the blood in the ICU (Intensive Care Unit) and up to 70% on hematology wards [10]. It is believed that this tendency is the result of the prophylactic use of azoles, especially fluconazole, which contributes to selection of naturally resistant species such as *C. krusei* and *C. glabrata*. In recent years, more and more iatrogenic fungal infections caused by fungi of the genus *Aspergillus*, *Rhizopus* and *Mucor* have been recorded [9]. Particularly vulnerable to fungal infections are patients with neoplastic diseases of the pharynx and larynx. The presence of mixed biofilm with the participation of bacteria and *C. albicans*, or fungi other than *C. albicans* (*C. krusei*, *C. tropicalis*), was found on the voice prostheses of patients undergoing total laryngectomy [11]. Other studies have examined the impact of mycological contamination of air in a hospital environment (patient room, hallway, toilet) on the fungal colonization of tracheotomy tubes in 13 cancer patients residing in the laryngological ward of the Department of Otolaryngology, Jagiellonian University Medical College in Cracow (A. Gniadek, P. Krzyściak, A. Hawryszuk, A.B. Macura, T. Brzostek, J. Składzień). From 105 tested samples of air the *A. fumigatus*, *A. flavus*, *A. ochraceus*, *A. niger*, *A. terreus*, *Mucor* sp., *Rhizopus* spp. and *Candida* spp. were most frequently isolated, while in tracheotomy tubes the non-albicans *Candida* strains were found. About 23% of the strains of mold fungi (*A. fumigatus* and *A. flavus*) were detected both in the air of the examined premises, as well as in tracheostomy tubes of patients.

Fungi and their spores easily spread in the environment. It has been well documented that airborne transmission of pathogenic fungi can occur when the air conditioning systems are used. Studies on the role of drinking water supply systems in the spread of fungi have been rare so far, and the results are questionable. The research conducted in Olsztyn and Ostrołęka revealed the presence of 17 species of fungi from 9 genera in tap drinking water and 13 species from 5 genera in bottled water – carbonated, non-carbonated and mineral waters (A. Biedunkiewicz, L. Schulz, K. Kowalska, K. Stojek, M. Dynowska, E. Ejdys, E. Sucharzewska, D. Kubiak – University of Warmia and Mazury in Olsztyn). Among the identified fungi there were also 3 species of the genus *Exophiala* (so-called „black yeast"): *E. spinifera, E. jeanselmei, E. castellani*. They are categorized to BSL-2 in biosafety classification, and therefore as potential pathogens. They cause phaeohyphomycetes, which are mainly responsible for subcutaneous infections. Fungi of the genus *Exophiala* have been identified in biofilms of tap aerators [12]. They can also develop in kitchen devices. „Black yeasts" were detected in 56% of

dishwashers tested in Europe, Americas, Africa, Asia and Australia [13]. Water pipes can be an important reservoir of fungi potentially pathogenic to humans. There is possibility that different fungi species are spreading with the tap water in the form of an aerosol. Studies conducted in Brazil showed the presence of *Fusarium solani* in tap water in hospital oncology department. The same fungus species was diagnosed as the cause of invasion in the department' patient with transplantation of hematopoietic stem cells [14].

Fungi commonly found in the environment are often associated with organisms living in it. Migration of wild birds, related to their reproductive cycle and/or the search for feeding grounds, may contribute to the spread of fungi potentially pathogenic for humans. The taxonomic diversity and prevalence of fungi present in selected parts of the respiratory and digestive tracts of cormorants, mallards and charadriiformes were evaluated. For the mentioned birds, Poland is a major migration corridor (M. Dynowska, W. Meissner, K. Jakubas, K. Góralska, A. Biedunkiewicz, D. Jakubas, J. Dziekońska-Rynko, J. Rokicki, E. Ejdys, E. Sucharzewska, D. Kubiak – University of Gdansk, University of Warmia and Mazury in Olsztyn, Medical University of Lodz). It has been shown that about 50% of healthy wild birds are reservoirs and vectors of fungi potentially pathogenic to humans. In 46% of birds, fungi were present in the beak and cloacae, i.a., the species of *Candida albicans*, *C. krusei*, *Cryptococcus neoformans*, *Cr. laurentii*, *Rhodotorula glutinis*, *Rh. rubra*, *Aspergillus fumigatus*, *and Trichosporon cutaneum*. Wild ducks often accompany humans and undergo synurbization. In the birds caught in cities areas (Gdansk, Olsztyn) and hunted, the presence of fungi was detected in 85.4% of swabs sampled from the beak and cloaca [15]. Isolation of fungi from cloaca suggests that fungi have passed through the digestive tract of a bird, thus indicating a carrier state. Wild birds may be a link in the epidemiological chain of fungal infections; they transmit potentially pathogenic fungi via the environment (water reservoirs) to the human.

The growing number of anthropozoonoses, especially in children, necessitates continuous monitoring of the health status of both the soil and the prevalence of intestinal parasitic infections in animals. Parasitological examination of soil conducted in places of children and youth recreation in Poland confirm the presence of egg parasites of dogs and cats, mainly of the genus *Toxocara*. It is estimated that in Europe from 1 to 4% of the adult population shows the presence of anti-*Toxocara* spp. antibodies and in the pediatric population the ratio is several times higher. Toxocarosis is considered to be the most commonly occurring helminthozoonosis in the developed countries [16]. Female worms of dogs and cats are highly fertile. It is estimated that a six-week puppy with the invasion of 12 females excretes about 300 thousand eggs per defecation (10 million per week). In humans as accidental hosts the adult *Toxocara* do not develop, which excludes the coproscopic diagnostics of this parasitosis. *Toxocara* larvae in the human body can live up to 10 years after infection while maintaining the ability to continue migration. The parasitosis in humans can occur in various forms – as a visceral larva migrans syndrome, ocular larva migrans syndrome, neurotoxocariasis, covert toxocarosis, and may be asymptomatic [16]. The team from the Medical University of Lodz (K. Szwabe, J. Błaszkowska, P. Kurnatowski, A. Wójcik, K. Góralska) assessed the status of Lodz recreational areas contamination with *Toxocara* eggs. Half of selected localities were well protected from access by animals. 528 soil samples from 11 playgrounds and 11 sandpits were examined by flotation method. The research material was collected in the autumn (2011) and spring (2012), both from the surface and deeper layers of the soil. A total of 46 eggs of *Toxocara* spp. were detected in the samples, of which 36.9% had a larva. Regardless of the season, the highest soil contamination with *Toxocara* eggs was found in the samples from unfenced playgrounds (15.8% of positive samples; density of eggs – 1.2 per 100 g of sample); in these locations six times higher contamination with eggs was reported as compared to secure places free of animals. In samples of sand taken from the enclosed sandpits a total of three eggs of *Toxocara* spp. were detected (average density of eggs – 0.1/100 g). The results confirm the fact that protection of children's playground from the access of animals significantly reduces contamination with developmental forms of intestinal parasites of dogs and cats. However, the sanitary condition of fenced playgrounds depends mainly on proper attitude of pet owners and users of these places of recreation.

In Europe, parasitic diseases transmitted by vectors are revealed mainly in individuals returning from endemic regions of such parasitoses occurrence. Of approximately 125 million people

traveling annually to malaria-endemic areas, about 10 000 cases of malaria are recorded after returning home [17]. In 2012, 16 locally acquired invasions were recorded in Greece, while 60 cases were imported by immigrants [18]. In the United States, each year, approximately 1,500 cases of malaria are diagnosed, especially in travelers [19]. In Poland, 35 cases of malaria were reported in 2010, 16 in 2011, 20 in 2012, and 31 cases until November 30, 2013 [20].

A case of malaria caused by *P. malariae* in a tourist after returning from Africa was presented by the team from Poznan (M. Kłudkowska, K. Frąckowiak, Ł. Pielok, K. Mrówka – University of Medical Sciences in Poznan, Laboratory of Parasitological Diagnostics of Heliodor Święcicki Clinical Hospital in Poznan). They have also presented, on the basis of their own long-term experience, the evaluation of the clinical course of malaria imported into Poland. Malaria is a common cause of fever requiring special differential diagnostic procedures for persons returning from endemic areas of the tropical and subtropical zones. The severity of clinical symptoms of malaria appearing most commonly after a trip to the countries of Equatorial Africa and a long-term stay (more than 2 weeks) correlates with the lack of pre-departure medical consultation and lack of appropriate pharmacological anti-malarial prophylaxis by patients. For the detection of *Plasmodium* spp. invasion still the „gold standard" is examination of thin and thick blood smears. Determination of the level of *P. falciparum* antigen, histidine-rich protein 2 (HRP-2) in the peripheral blood, has an important clinical and prognostic value for the assessment of the severity of the clinical course of malaria, determination of the risk of multiple organ failure and monitoring the effectiveness of antiparasitic treatment.

Leishmaniosis is caused by protozoa of the genus *Leishmania* transmitted to humans by the bite of flies of the subfamily Phlebotominae. More than 90% of 200-400 thousand cases of visceral leishmaniosis (VL) worldwide are the most severe forms of the disease occurring in India, Bangladesh, Sudan, Ethiopia, and Brazil. Cutaneous leishmaniosis (CL), estimated at approximately 0.7–1.2 million cases a year, is widespread in Latin America, the Mediterranean, the Middle East and Central Asia [21]. VL is endemic in 9 countries of the European Union, with the incidence of 410–620 cases per year (2003–2008, WHO data). CL also occurs in the same regions, but with much greater frequency. In Southern Europe, zoonotic VL and CL caused by *L. infantum* species, having dogs as the reservoir host and antroponotic CL caused by *L. tropica*, sporadically occurring in Greece are recorded. A third parasite species, *L. donovani* (considered as antroponotic), has been reported in Cyprus, where it causes both VL and CL. The case of leishmaniosis in Poland diagnosed and treated in Poznan, concerned a patient who had spent two months in Croatia and previously 14 months in Greece (M. Paul, J. Stefaniak – Poznan University of Medical Sciences). A systemic inflammatory response syndrome (SIRS) with organ failure and concomitant insulin-dependent diabetes mellitus was diagnosed; immunodiagnostic tests detected the presence of specific IgG antibodies against antigens of *Leishmania* spp. The bone marrow bioptate revealed amastigote forms of *Leishmania* spp.

Of note, in Poland, in addition to tourists, especially endangered with parasitic diseases transmitted by vectors can be truck drivers and employees of construction companies returning from the Middle East, missionaries, and persons involved in the field work in India and Sudan, and soldiers of stabilization missions (Iraq, Afghanistan, Congo, and Chad). After returning from countries of different climate zones it is advisable to perform medical check-ups for the detection of diseases in tropical medicine reference centers. Most diseases contracted in tropical and subtropical zones may be asymptomatic or oligosymptomatic, and clinically manifest a few months or even years after returning.

Endemic or potentially endemic parasitosis in 30 countries on all continents is human dicrocoeliosis – a zoonosis caused by the presence of *Dicrocoelium dendriticum* in the bile duct. The definitive hosts of this parasite are: sheep, goats, cattle, horses, deer, hares, rabbits, and occasionally humans. Accidental eating of an infected ant (second intermediate host) may be the cause of parasitosis (with symptoms of inflammatory foci in the bile duct, obstruction, jaundice, liver enlargement, and the detection of eggs in stool). Pseudodicrocoeliosis is also possible in humans after consuming raw or undercooked liver of an infected animal (no symptoms, eggs in the feces for a short period). Data on sporadic cases of human dicrocoeliosis have been presented (A. Okulewicz, I. Kamoń – University of Wroclaw). In 2004 two cases were found in Germany: in an emigrant from Afghanistan and in a tourist 3.5 years after returning from Morocco, in 2010 in Turkey –

one case, and in 2011 cases of dicrocoeliosis in a farmer family from Egypt detected simultaneously with parasitosis of domestic animals were described [22–24]. There were also cases of pseudodicrocoeliosis recorded in 2011 in Kyrgyzstan in 11 children (8% of the examined) aged 2–15 years staying in poor hygienic and social conditions, and in 2008 a case of pseudodicrocoeliosis in a patient with Crohn's disease [25,26].

Babesiosis is a disease caused by a parasitic protozoan transmitted by vectors – ticks of the species *Ixodes ricinus* (in Europe) and *I. scapularis* (in the USA). Approximately 110 of *Babesia* species, pathogenic for a wide range of vertebrates, are known. Human pathogens are *B. microti* (detected in the United States and Japan), *B. divergens* (Europe), *B. duncani*, *B. venatorum* (strains: WA1, CO1, EU1) and, detected in individual cases, *B. bovis* (in the former Yugoslavia and Spain) also *B. canis* (in France). In the study on ticks inhabiting the eastern part of Poland *Babesia* species pathogenic to humans and animals were identified. The samples collected from ticks of Kielce and Lublin regions revealed the presence of genetic material of the species *B. venatorum* – genotype EU1 (W. Rożej-Bielicka, A. Masny, E. Gołąb – Department of Medical Parasitology NIPH-NIH, Warsaw). The research on arachnids collected in the forests of the Lublin region demonstrated that the tick species *Dermacentor reticulatus* may also be a potential vector of *B. microti* [27]. Transmission of the protozoan into the human body most frequently occurs through the bite of an infected tick. It can also be transferred by blood transfusion from an infected person and transplacental or perinatal invasion [28].

Infections acquired in a hospital environment may be caused by viruses, bacteria, fungi and parasites. The most common pathogens in nosocomial infections in intensive care units are bacteria: *Staphylococcus aureus* (14.3%), *Pseudomonas* spp. (13.9%), *Escherichia coli* (11.2%), *Klebsiella pneumoniae* (8.8%), and fungi of the genus *Candida* (11.9%). In Poland most infections result from improper hospitals` sanitary conditions, which were found in 22.5% of the inspected hospitals (Report of the Chief Sanitary Inspectorate, 2012). Of particular importance are anomalies in the central sterilizing services units – 77% of the evaluated hospitals did not satisfy the requirements of the Ministry of Health. The data on environmental conditioning of fungal and parasitic nosocomial infections were presented by the team from Lodz (K. Góralska, P. Kurnatowski – Medical University of Lodz). Evaluation of insects' occurrence in the hospital environment revealed cockroaches in 46% of hospitals, mainly in the kitchens, utility and storage areas. The insects are mechanical carriers of numerous microorganisms: 48.9% of cockroaches carry fungi of the genus *Candida*, 22.6% – bacteria of the genus *Enterobacter*, 21% – *Klebsiella*, 17.3% – *Enterococcus*, 16.5% – *Staphylococcus*. Mosquitoes were present in 4%, while flies in 35% of hospitals. It should be drawn that some patients, especially immobilized, with extensive damage to the skin and soft tissue, neuropathic complications (diabetic foot), the mentally ill, or with autism may be exposed to myioses. The presence of fungi in the air of operating rooms during surgical procedures was found in over 56% of them. For the spreading of fungi, hands of medical personnel are of a great importance: fungal contamination was detected on 5–47% of hands. Only 30% of medical staff wash their hands before and after contact with the patient, only 46% use gloves, and as many as 73% wear jewelry in the workplace. The threat for the patient may be water and food, and also other patients. A separate problem is the blood and organ transplants from an infected donor. For medical and laboratory personnel, the risk is connected with pathogens transmitted through blood and body fluids, including parasitic protozoa of the gastrointestinal tract, blood, tissues, and helminths.

Recently hirudotherapy is becoming an increasingly common tool of natural medicine supporting treatment of many diseases such as high blood pressure, peripheral vascular disease, hemorrhagic diathesis, rheumatic disease. Its use in 60–80% determines the success of replantation and reconstructive surgery due to the effective prevention of thromboembolic complications [29]. In June 2004, the U.S. Food and Drug Administration (FDA) recognized the medicinal leeches as „therapeutic agent". Since then hirudotherapy has appeared in the International Classification of Medical Procedures (ICD-9, No. 99.991). However, attaching leeches can cause many adverse effects: prolonged bleeding from wounds, inflammatory connective tissue disease, local allergic reaction or infection with pathogens. The possibilities of infectious complications after hirudotherapy have been presented by the team from Lodz (A. Litwinowicz, J. Błaszkowska –

Medical University of Lodz). Most frequently local infections with symbiotic bacteria of the genus *Aeromonas* are recorded, which requires implementation of antibacterial therapy in patients. From the gastrointestinal tract of *Hirudo medicinalis* leeches intended for medical purposes researchers isolated a number of opportunistic species which are common etiologic agents of nosocomial infections (*Morganella morganii*, *Klebsiella pneumoniae*, *Providencia alcalifaciens*, *Pseudomonas* sp., *Serratia* sp.). Moreover, decontamination of the animals with 0.02% chlorhexidine solution is not very effective, and the use of other antiseptics leads to loss of willingness of sucking blood by leeches [29]. The transmission of microorganisms from a leech to a patient may occur during contact with the surface of the body and jaws of leeches, while sucking blood, and also by the return flow of blood from the leech's crop during its detachment. Transmission of viruses (HBV, HIV), bacteria, fungi and even protozoa may occur at the time of blood feeding [30]. The survival of microorganisms in the gastrointestinal tract of *Hirudo medicinalis* was experimentally evaluated. It was found that the human and animal viruses and bacteria (*Staphylococcus aureus*, *Escherichia coli*) can survive in the intestine up to 6 months, and protozoa (*Toxoplasma gondii*, *Trypanosoma brucei brucei*, *Plasmodium berghei*) for 5–6 weeks, indicating the possibility of multiplication and continuation of development in the leech's intestine [31]. These experiments confirmed that medicinal leeches, having contact with the blood of the host, are potential vectors of various pathogens.

Scabies is the most common parasitic disease of the skin caused by *Sarcoptes scabiei* var. *hominis*. The World Health Organization estimates that there are more than 300 million cases of scabies worldwide each year. In Poland the majority of cases were registered in 1968 (186 956), while in 2004–2008 the number of annually reported cases of scabies ranged from 12,500 to over 11,000. Currently in Poland (since 01.01.2009) it is difficult to estimate the prevalence of this parasitic mite in the absence of any obligation to report diagnosed cases of scabies to sanitary and epidemiological stations. Skin lesions in the course of this parasitosis are varied and dependent on local and general immune response. Immunocompromised patients develop a specific form of scabies, called hyperkeratotic scabies (Norwegian scabies) with a severe clinical course and occupation of large areas of the body. In people who care about the hygiene the symptoms are less pronounced: separate lesions in the form of isolated clumps (scabies of the cleanly). Particularly noteworthy is the problem of infection of medical staff with *S. scabiei* (A. Kwapiszewska, A. Jagłowska, P. Kozarzewski, K. Góralska – Medical University of Lodz). Scabies is the most common parasitosis reported by health services staff, mainly in intensive care units, rehabilitation centers, infectious diseases and internal medicine wards, and emergency services. Infections among nurses, physiotherapists, doctors and the staff in direct contact with AIDS patients have been most frequently recorded. Duration of hospital outbreaks of scabies epidemic is estimated at average 14.5 weeks (5–52 weeks). The mean percentage of infected health care workers was estimated at 34.6% (6.95–88%) [32]. The average number of infected employees per outbreak was 18 individuals (range 3–82). The main causes of scabies outbreaks among medical staff are: immunosuppressive therapy frequently used in patients, resistance of *S. scabiei* to scabicides, as well as failure to comply with hygiene. The problem with a constant spread of scabies infection is mainly due to failure to diagnose the disease and inadequate or ineffective treatment.

Dermatophytes of the genera *Microsporum*, *Trichophyton* and *Epidermophyton* are the major etiological agent of superficial fungal infections. Classic identification of these fungi species is based on observations of micro- and macroscopic axenic strains isolated from biological material. Because of slow rate of dermatophytes growth, the differentiation is possible only after 2–3 weeks following sampling. Modern diagnostics of dermatophytes uses Polymerase Chain Reaction and its variants: Real time PCR, PCR fingerprinting, RAPD (Random Amplification of Polymorphic DNA), PCR/RFLP (PCR and Restriction Fragment Length Polymorphism), AP-PCR (Arbitrarily Primed PCR). Molecular diagnostic methods do not require culture of the pathogen, but the characteristic structure of the cell wall of dermatophytes does not allow the use of most commercial DNA isolation kits directly from clinical specimens. The new method that enables identification of pathogens is mass spectrometry with desorption/ionization laser – Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (I. Dąbrowska – Warsaw Agriculture University). The technique

based on a unique protein profile analysis allows the identification of bacteria and fungi on the basis of molecular weight fragments of their specific, mainly ribosomal proteins. Comparison of protein profile of the tested pathogen with standard set of reference microbial proteins can determine the species in cultures obtained from biological and environmental samples [33]. The technique is also helpful in differentiating species of dermatophytes. Nenoff et al. (2013) showed 99.3% species similarity of dermatophytes strains determined by PCR and MALDI-TOF MS. Only two isolates of *Trichophyton violaceum* in all 285 tested were not identified by this method [34].

Of the 12 species of *Trichinella* described in the world, four are found in Europe: *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis* [35]. In Poland, the presence of two species was confirmed in domestic and wild animals: *T. spiralis* and *T. britovi*. *Trichinella* transmission takes place in synanthropic (household), sylvatic (forest) or synanthropo-sylvatic environments. *T. spiralis* is the most pathogenic species for humans and the major etiological agent of most cases of trichinosis in man. There have been reports of human infections with other *Trichinella* species, including *T. britovi*. The diagnosis of *Trichinella* infection is commonly carried out based on the results of the ELISA immunoenzymatic assay which allows detection of specific antibodies in serum. Previous serological methods (ELISA, Western Blot) used in the diagnostics of trichinosis do not allow determination of *Trichinella* species. This is due to the lack of effective and species-specific antigen that guarantees high reproducibility and comparability of results. Information on the common antigens, as well as the ones specific for different *Trichinella* species, is extremely valuable and useful in the development of so-called species-specific diagnostics. The team from the Institute of Parasitology, Polish Academy of Sciences (J. Bień, B. Moskwa, W. Cabaj – PAS, Warsaw) analyzed the protein profiles of excretory/secretory (E/S) antigens of *T. spiralis* and *T. britovi* muscle larvae and identification of species-specific immunoreactive proteins using two-dimensional fluorescence differential electrophoresis (2D-DIGE), liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and immunoblot. The analysis of 2-D DIGE and 2-D immunoblot showed significant differences in the protein profiles of E-S material of *T. spiralis* and *T. britovi* and revealed the presence of species-specific proteins/antigens that can be used in the trichinosis diagnostics. The obtained results highlight the usefulness of the 2-D DIGE method as a tool used to compare the proteomes of different *Trichinella* species [36].

*Toxoplasma gondii* as a „successful parasite", which is continuously showing new faces and causes controversy in researchers, was presented by H. Długońska (University of Lodz). The chronic phase of toxoplasmosis infection is almost asymptomatic, but not without consequences for the host. Tissue cysts of the parasite preferentially localize in the brain, muscles and eyes, and usually persist there to the end of the host life. Infected by the parasite mice have shown changes in activity, learning ability and memory, loss of fear against natural enemies (which promotes the transfer of the parasite to the definitive host), as well as a decrease in the level of thyroxin (T4) in serum, abnormalities in pulsatile TSH secretion from the pituitary, changing profile of the immune response (instead of antibodies class IgG1 - IgG2). In humans altered behavior has also been observed: a positive correlation between duration of infection and decrease in the strength of superego (moral and learned standards controlling instincts and drives), lower IQ, poorer educational attainment. In the infected group there are more patients with schizophrenia and epilepsy than in the healthy group; higher mortality is noted in the infected persons with brain tumors, and also higher suicide rate. *T. gondii* cysts colonize the perikaryon, dendrites and axon of neurons. Proteins of the parasite tachyzoites interfere in the infected neurons with signaling pathway stimulated by glutamate, which results in hypo- or hyper-responsiveness [37]. An increase in the number of tachyzoites in human fibroblasts and neonatal rat astrocytes after adding dopamine neurotransmitter to the culture was also noted [38]. The protozoan „inoculates" the host cells, neurons of the brain and immune cells with its antigens from rhoptry [39]. The phenomena may indicate a manipulation by the parasite with cells and the host immune response.

Prolactin (PRL) is a pituitary tropic hormone playing key role in innate immunity. It induces phagocytosis mechanisms of macrophages, microglial cells and other non-phagocytic cells (epithelial cells). It is involved in the production of proinflammatory cytokines and NO, which are necessary for the recruitment of leukocytes to the

site of infection. PRL may also modulate leukocyte adhesion to the endothelium. The protective effect of prolactin (PRL) on the infection with intracellular protozoan *Toxoplasma gondii* in an experimental model of toxoplasmosis in mice was described [40]. In addition, a correlation between the frequency of *T. gondii* infection and serum PRL levels in the human population was found. The literature data show that hyperprolactinemia condition may inhibit the development of infection caused by *T. gondii.* It has been shown that PRL deficiency increases susceptibility to infection with intracellular protozoa such as *T. gondii*, *P. falciparum*, *T. cruzi*, *Leishmania* sp. In pregnant women infected with *P. falciparum* low levels of PRL and a negative correlation between PRL and IL-4 and IL-10 were reported [41]. In pregnant animals the protective effect of the hormone in relation to the developing embryo was also observed. *Neospora*-seropositive cows in which there was no miscarriage had higher PRL concentrations in plasma than seronegative animals. The concentration of PRL after miscarriage decreased, indicating a protective effect of the hormone [42]. Researchers from Lodz University (K. Dzitko, B. Dziadek, J. Gatkowska, H. Długońska) examined live tachyzoites of *T. gondii* belonging to different strains: RH (genotype I) and ME 49 (genotype II) for binding human and sheep prolactin. Using two different tests, cellular ELISA and direct immunofluorescence assays, they demonstrated the capacity of these strains of *T. gondii* tachyzoites to bind human and sheep prolactin. It is a specific binding depending on the concentration of PRL. Additionally, it was also shown that the *T. gondii* cell lysate derived from tachyzoites of *T. gondii* strains RH and BK (genotype I) binds specifically the sheep and human PRL.

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