

## Review articles

## Current problems concerning Parasitology and Mycology with regard to diseases of the skin and its appendages<sup>1</sup>

Joanna Błaszowska, Anna Wójcik

Chair of Biology and Medical Parasitology, Medical University of Lodz, 1 Hallera Square, 90-647 Lodz, Poland

Corresponding author: Joanna Błaszowska; E-mail: joanna.blaszowska@umed.lodz.pl

**ABSTRACT.** Current issues concerning Parasitology and Mycology with regard to diseases of the skin and its appendages are presented. Aspects of diagnostics, clinical picture and therapy of skin and nail mycoses, as well as difficulties in the diagnosis and treatment of both native parasitoses (toxoplasmosis) and imported human tropical parasitoses (malaria, filariasis) have been emphasised. The clinical importance of environmental mould fungi in nosocomial infections and fungal meningitis, as well as selected properties of fungi isolated from patients with head and neck neoplasms treated by radiotherapy are discussed. Other mycological topics include the characteristics of newly-synthesized thiosemicarbazides and thiadiazoles as potential drugs against toxoplasmosis and their biological activity against *Toxoplasma gondii* tachyzoites, selected molecular mechanisms of resistance to azoles, *Candida albicans* strains and a new tool (barcoding DNA) for describing the biodiversity of potential allergenic molds. The importance of environmental factors in pathogenesis of mycoses and parasitoses is noted. The characteristics of pathogenic fungi isolated from natural ponds in Bialystok and potentially pathogenic yeast-like fungi isolated from children's recreation areas in Lodz are presented. The ongoing problem of anthroponoses is considered, as are the roles of stray cats and dogs in contaminating soil with the developing forms of intestinal parasites. The characteristics of the human microbiome, including population composition, activity and their importance in normal human physiology, are presented, as are the major goals of the Human Microbiome Project initiated by National Institutes of Health (NIH).

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

During the last decades, a permanent increase in the number of fungal skin infections has been observed worldwide. The high prevalence of superficial mycoses shows that approximately 20–25% of the world's population has skin lesions, making these one of the most frequent forms of infection. Pathogens responsible for those diseases are primarily geophilic, anthropophilic and zoophilic dermatophytes from the genera *Trichophyton*, *Microsporum* and *Epidermophyton*. There appears to be considerable inter and intra-continental variability in the global incidence of these fungal infections. Dermatophytes are keratinophilic and keratinolytic fungi, characterized by a high affinity to keratin-containing tissues. In everyday clinical practice they are responsible for superficial mycoses of skin (tinea faciei, tinea barbae, tinea corporis, tinea cruris, tinea manuum or tinea pedis), nails (onychomycosis, tinea unguium) and hair (tinea capitis) [1,2].

The spectrum of dermatophytes isolated from skin lesions has changed in the last 70 years in most European countries. *Microsporum audouinii* and *Epidermophyton floccosum*, ranked highest before the Second World War in Germany, have been replaced by *Trichophyton rubrum*, which nowadays accounts for 80–90% of the strains, followed by *T. mentagrophytes*. This trend, also observed in the Central and Northern Europe, is directly connected with the increased incidence of tinea pedis. In contrast, in Southern Europe and in Arabic countries, zoophilic dermatophytes, such as *Microsporum canis* or *Trichophyton verrucosum*, are the most frequently isolated. In Europe, especially in Mediterranean countries, the incidence of *M. canis* infection has strongly increased during the recent years and this dermatophyte is now the most prevalent in tinea capitis in children [3].

Infections caused by dermatophytes have become a very serious problem, not only a clinical

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one, but also with epidemiological and therapeutic aspects. Clinical manifestations of mycoses vary depending on the site of infestation and the type of strain and therefore, accurate identification of the strain is crucial in order to facilitate rapid treatment and to prevent spread of the disease. Skin mycoses often present a typical clinical picture, however, in some cases they may mimic other disorders, such as pityriasis rosea Gibert, granuloma annulare, sarcoidosis, urticaria, erythema annulare centrifugum, lupus erythematosus (especially subacute cutaneous form – SCLE), erythema chronicum migrans, erythema multiforme, psoriasis, eczema and contact dermatitis. Also, the previous application of some drugs (such as steroids, antihistamines, antibiotics or zinc ointment) may modify the presentation of dermatomycoses in some patients. Therefore, it is of great importance to collect scrapings for microscopy and culture before starting treatment with oral or topical antifungals (J. Narbutt; Medical University of Lodz).

The commonest risk factors for fungal skin infections are: antibiotic and corticosteroid use, a depressed immune system due to chemotherapy and HIV infection, the coexistence of diabetes, as well as environmental factors like increased moisture, lifestyle, contact with soil, form of recreation and taking part in different sports and, in some individuals, a genetic predisposition towards mycoses. Religious ceremonies and traditions, like ritual ablutions, are also of the great importance. The relative occurrence of the etiologic agents and fungal skin infections varies from country to country and from one climatic region to another. In tropical countries, a warm and humid climate, crowded living and poor sanitary conditions all promote the spread of mycoses. It is worth noting that mycoses of the skin covering the trunk and face have been more frequently observed in those countries, whereas in a moderate climate, fungi mostly affect the feet and hands, particularly the nails. In Lodz (Poland), a higher prevalence of skin and nail lesions was noticed in middle-aged and elderly patients as compared to young subjects, with significantly greater occurrence on the hands of women than men (J. Kwaśniewska, E. Szefer, A. Jaskółowska; Medical University of Lodz). Mycosis of the large toenail was most frequently diagnosed, while infection of the remaining toenails, foot skin and spaces between toes occurs more rarely. In the majority of cases, mycosis of the

smaller toenails was induced by *T. mentagrophytes* and *T. rubrum*, whereas mycosis of the large toenail was often associated with *Candida* species and also moulds of genera *Fusarium*, *Alternaria*, *Aspergillus*, *Scopulariopsis* [4]. Interestingly enough, *S. brevicaulis*, a common saprophytic fungus found in soil, on vegetables and other organic waste, having a wide geographic distribution, may be an etiological factor of skin lesions in various locations and also generalized mycoses. Several case reports have described *S. brevicaulis* infections of deep tissue or skin in immunocompromised hosts and there have been sporadic cases of ocular infection. A case of slowly progressive granulomatous face skin infection in an otherwise healthy person has also been found [5].

Onychomycosis, infection of the nail plates, is caused by dermatophytes, nondermatophytes moulds or yeast species. In the majority of patients, onychomycosis is viewed as more than a mere cosmetic problem. In spite of improved personal hygiene and living environment, nail mycoses continue to spread and persist. It has been estimated that nail mycoses may concern nearly 30% of adults in Europe, whereas their prevalence in Australia and the United States accounts for 10% [6].

Numerous studies have revealed that nearly 10% of the cases of onychomycosis are caused by nondermatophyte molds (NDM): *Scopulariopsis brevicaulis*, *Aspergillus* spp., *Acremonium* spp., *Fusarium* sp., *Onychocola canadensis*, *Natrasia mangiferae*, *Alternaria* spp., *Scybalidium dimidiatum*, *S. hyalinum*, *Geotrichum candidum* or *Cladosporium carrionii*. Unlike dermatophytes, those fungi are not keratolytic and can only invade a nail plate previously destroyed due to trauma, dermatophyte activity or another nail disease. For this reason, the incidence of onychomycosis due to NDM is estimated from 1.45 to 17.6% [7,8].

Keratinophilic molds may be either contaminants of biological material in the laboratory or pathogens causing nail lesions, which may create serious problems for the correct diagnosis of NDM onychomycosis. Moreover, diagnostic criteria are inconsistent between mycological laboratories. Based on several studies, six major diagnostic criteria were identified: identification of the NDM in the nail by microscopy, the presence of mycelium elements (using a potassium hydroxide preparation – KOH with Parker ink or Calcofluor White; alternatively KOH may be replaced by Na<sub>2</sub>S or NaOH), isolation

in culture, repeated isolation in culture in different time intervals, quantitative evaluation of inoculum, failure to isolate a dermatophyte in culture and histology (periodic acid Schiff). Most studies recommend using at least three of those criteria to rule out contamination, always including the KOH preparation for direct microscopy and isolation of the organism in culture (P. Krzyściak; Jagiellonian University-Medical College). Of note, mixed nail invasions are possible and dermatophytes may not be isolated till the second or the third culture. If the same mould is still present in subsequent cultures, it should be regarded as the etiological factor of onychomycosis [9].

Fungi from the genus *Candida*, although uncommon, may be etiological factors for meningitis. Mostly the disease affects immunosuppressed patients, those treated with broad-spectrum antibiotics or those receiving parenteral nutrition, or it can be the result of disseminated disease. In addition, two specific patient groups, premature neonates and neurosurgical patients, are at increased risk. Several studies based on autopsies have proved that the true prevalence of fungal meningitis can be underestimated in everyday clinical practice. Fungal infection of the central nervous system manifests as meningitis, micro or macro-abscesses, vascular and medullar injury. *C. albicans* accounts for 70–100% of all fungal isolates, but an increase in the occurrence of other species, such as *C. glabrata*, *C. tropicalis*, *C. parapsilopsis* and *C. lusitaniae*, has been observed. Infections caused by non albicans species, are frequently resistant to antifungals and are associated with higher mortality. In contrast to meningitis caused by *C. albicans*, *C. tropicalis* meningitis has been increasingly reported in adults. Most cases of *C. tropicalis* are postoperative complications of head and neck surgery, including mastoid exploration, craniotomy, and ventricular-peritoneal shunt. The diagnosis of meningitis is established by repeated positive CSF cultures. CSF parameters are variable, with a mild lymphocytic or polymorphonuclear pleocytosis and an increased protein level. Fungal elements are generally not seen. Hence, CSF abnormalities are indistinguishable from cryptococcal, tuberculous, and some bacterial meningitides. It is of great concern that despite appropriate treatment, this entity is still associated with 10–30% mortality, and the percentage of neurological sequels ranges from 18 to 29%, which highlights the importance of timely recognition and

management of *Candida* related CNS infections [10].

A unique case of fungal meningitis with many years' course, probably stemming from a communication injury, is also described. The patient was repeatedly hospitalized between 2006 and 2012 in the Department of Infectious Diseases in response to a fever above 38°C, headache, nausea and vomiting. The patient was discharged several times from the hospital on his own request before finishing the diagnostic and therapeutic procedures. In this case, *C. glabrata* was found to be responsible for the meningitis; it was also isolated from the nasopharyngeal cavity with a negative culture from urine and feces specimens. The most reliable explanation is that fungal meningitis is connected with a previous injury and untreated sinusitis, which eventually leads to quantitative changes in the T lymphocyte CD4 count and the spread of *C. glabrata* from nasopharynx towards the central nervous system. It is recommended that every lymphocytic meningitis of unknown origin should be carefully screened for fungal etiology, especially in case of depressed cellular immunity (A. Michowicz, M. Jabłkowski; Medical University of Lodz).

During the last decade, invasive infections caused by *Candida albicans* have been revealed as an increasing problem especially in immunocompromised patients. Of many antifungal agents, only very few substances are established as agents in the treatment of systemic fungal infections. Azoles, especially fluconazole, have been used as first-line antimycotics for the prophylaxis and treatment of fungal infections. As the number of patients treated with fluconazole has increased over the last 15 years, azole resistance has emerged as a major problem. The azoles bind to and inhibit the activity of lanosterol 14 $\alpha$ -demethylase (Erg11p), a key enzyme in the fungal ergosterol biosynthesis pathway. The development of clinically resistant *Candida* strains can occur by several mechanisms, including infection with intrinsically resistant organisms, selection of resistant organisms secondary to antifungal drug pressure, and development of resistance in a previously susceptible organism. Several mechanisms of resistance to azoles have been described in *C. albicans* [11]. These include increased expression of drug efflux pump genes such as MDR1, CDR1 and CDR2, amino acid substitutions in the target enzyme Erg11p due to mutations in the ERG11

gene, as well as overexpression of ERG11 [12]. Overexpression of efflux pump genes such as MDR1 and MDR2 leads to a decreased intracellular azole concentration. In addition, mutations in the ERG11 gene encoding the target enzyme lanosterol 14 $\alpha$ -demethylase prevent the binding of azoles to the active site.

In a presented study by a team from the Medical University of Silesia (K. Gołabek, J. Strzelczyk, A. Owczarek, A. Wiczowski), the levels of expression of genes encoding ERG11 and efflux transporters (MDR1 and CDR1, CDR2) were monitored in susceptible and resistant isolates of *C. albicans*. A range of samples comprising 120 strains of *C. albicans* was isolated from hospitalized patients in the Independent Public Hospital in Nowy Targ. The antifungal susceptibility of *C. albicans* isolates samples to fluconazole, itraconazole and voriconazole was evaluated using ATB fungus2 (bioMérieux). Total RNA was extracted from *C. albicans* cells by use of the RNeasy® Mini Kit (Qiagen, Germany) and was characterised with the RNA LabChip Kit by 2100 Bioanalyzer (Agilent Technologies, USA). A quantitative analysis of the level of expression of the examined genes was performed by RT-Q-PCR. In this study, increased expression of the MDR1 and CDR2 genes was discovered in resistant *Candida* strains. Significant differences were observed regarding ERG11 expression in the tested azole-resistant and azole-sensitive strains of *Candida*. These results suggest that overexpression of MDR1, CDR2 and ERG11 genes can be an important mechanism of azole resistance in *C. albicans* clinical isolates.

Hospital-acquired infections can be caused by viruses, bacteria, fungi or parasites. Among the various fungi which may cause nosocomial infections, the most common are mould species, which mostly infect immunosuppressed individuals (A. Gniadek, A.B. Macura; Jagiellonian University-Medical College). It was estimated that the incidence of nosocomial infections varies between 5 and 10% with mortality calculated approximately to be 11%. The wider use of more aggressive methods of treatments, such as chemotherapy, radiotherapy, organ transplantation, long term administration of corticosteroids and immunosuppressive therapy promote nosocomial infections; fungal infections represent approximately 9–10% of all nosocomial infections. It is common knowledge that *Candida* species are the most common fungal pathogens causing nosocomial infections (85% of all mycosis)

whereas infections due to *Aspergillus* spp. account for 1.3%. However, the epidemiology has changed dramatically in recent years; invasive fungal infections caused by moulds, predominantly *Aspergillus* species, have increased substantially and newly emerging fungal pathogens such as Zygomycetes (e.g. *Rhizopus*, *Mucor*, *Absidia*), hyaline moulds (e.g. *Fusarium*) and other opportunistic species (e.g. *Scedosporium*) are increasingly being reported [13,14]. According to studies conducted on patients with hematologic malignancies during a 15-year period (1989–2003) a high prevalence of invasive fungal infection (31%) was found at autopsy, and although 77% of patient deaths were related to infection, only one-third of these infections were diagnosed by the accepted criteria before the patients died. Several cancer centers have reported an increase in the incidence of infections caused by difficult-to-treat opportunistic molds such as *Zygomycetes*, *Fusarium*, and *Scedosporium* species. Moreover, *Trichoderma longibrachiatum* was recently described as an emerging pathogen in immunocompromised patients, specifically in a renal transplant recipient [15]. An invasive mould infection is associated with a high rate of mortality. Also, mould fungi from *Microascales* (*Scopulariopsis brevicaulis*, *Pseudallescheria boydii* and *Scedosporium prolificans*) are the cause of nosocomial infections. Aspergillosis is an important cause of morbidity and mortality, particularly in allogeneic transplant recipients and neutropenic patients with hematologic malignancies. More than 90% of fusariosis cases have been reported in neutropenic patients with hematologic malignancies. Fungemia with *Scedosporium* sp. was noted in 11% of solid organ transplant recipients [16]. A potential reservoir of airborne moulds is the indoor air of hospital buildings. Disease can be transmitted by the inhalation of spores in air, consumption of contaminated water and by direct inoculation on disrupted skin or mucosa. Sporangiospores released by moulds range from 2.5 to 40  $\mu\text{m}$  in diameter, are easily aerosolized, and are readily dispersed throughout the environment. Respiratory tract colonization due to inhalation of the mould depends on the size of the spores. Large spores of *Alternaria* (23–34  $\mu\text{m}$ ) settle in the nosopharynx, but very small spores (*Rhizopus* 4–6  $\mu\text{m}$ , *Mucor* 4–8  $\mu\text{m}$ , *A. fumigatus* 2.5–3  $\mu\text{m}$ , *Fusarium* 2.4–3.5  $\mu\text{m}$ ) can reach the lungs and enter the alveoli. Hospital building works, failing to clean airflow systems and high-efficiency particulate air

(HEPA) filters increase the risk of invasive mould infections.

Radiotherapy is one method of treatment used in cancer therapy which can injure the oral, pharyngeal or laryngeal mucosa, and predispose the tissue to the development of fungal infections [17]. As seen from the literature data, the prevalence of fungal infection has increased in patients treated by chemo- and radiotherapy, and it may affect as many as 3/4 of the patients involved [18]. A determination of the frequency of fungal infection in particular phases of radiotherapy, and selected pathogenicity parameters of the strains has great significance in treatment and prognosis. In a study conducted by Moqbil and Kurnatowski (Medical University of Lodz), forty-three patients with oral, pharyngeal or laryngeal carcinoma were subjected to mycological examinations at four points during a course of radiotherapy: prior to the course, in week 3, on the last day of the course and 3 weeks after treatment. The fungal strains were isolated and identified from the oral cavity of these patients. A number of phenotypic characteristics, such as the activity of fungal hydrolytic enzymes, adherence properties of strains to epithelial cells and sensitivity to antifungal drugs, were determined. The presence of fungi in the mouth and throat was found in over 2/3, 4/5, about 3/5 and all of the patients in the first, the second, the third and the final examination, respectively. The most frequent species isolated from the patients was *Candida albicans* (40–60%) and multidrug-resistant *C. glabrata*. The isolated strains were the most sensitive to nystatine and miconazole, and in the least sensitive to ketoconazole and fluconazole. Regarding the particular biotypes for different species of *Candida*, the most significant diversity (classification for A–D biotypes) was demonstrated for *C. albicans* and *C. glabrata* [19]. The isolated strains are characterized by the highest activity of leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Considering the enzymes produced, most of the strains can be included to biotypes D<sub>3</sub> (22.5%), C<sub>6</sub> (17.5%) and A (15.0%) and less frequently to C<sub>3</sub> (7.5%), B<sub>1</sub> (7.5%) and B<sub>2</sub> (1.3%). The adherence ability was found to range from 0 to 57 fungal cells to one cell of the mouth epithelium. Fungal cells demonstrated increasing adherence to the epithelial cells of the mouth in the three subsequent batches (prior to, week 3, last day of radiotherapy). The high prevalence of fungi revealed in this study in the mouth and throat of

patients treated by radiotherapy emphasises the necessity to perform mycological examinations in this group of patients.

Endocrinopathies and coexisting mycoses concern not only humans: animals can also be affected. In recent years, an increasing frequency of fungal infections in cats and dogs has been observed [20,21]. The likely predisposition to infection was thought to be local or systemic immune compromise. It was revealed that *Candida albicans*, *C. guilliermondi*, *C. humicola* and *M. pachydermatis* were isolated more frequently from dogs and cats with diabetes, hypothyroidism and Cushing disease compared with healthy animals. All isolated samples were obtained from the oral cavity and rectum (W. Dardzińska, B. Dworecka-Kaszak; Warsaw University of Life Sciences).

Although much biological research depends upon species diagnoses, there is a considerable crisis in taxonomic research at the moment. It is believed that the sole prospect for a sustainable means of identification lies in the construction of systems that employ DNA sequences as taxon „barcodes”. The standard methods used in mycological diagnostics, based on phenotypic features, are characterized by 70–85% sensitivity and specificity, implying that almost 15–30% of species could not be correctly identified. This situation may especially concern *Candida non-albicans* species involved in the increasing number of various, sometimes generalized, infections which are resistant to standard antifungals. Therefore, molecular methods including barcoding may help to precisely identify the fungus. Nevertheless, it should be remembered that molecular methods, such as PCR with specific and universal primers, analysis of the length of PCR product, restriction fragment length analysis, hybridization with specific probes and DNA sequence analysis also have their limitations and always have to be preceded by basic procedures used in all mycological laboratories [22].

The ideal DNA barcode characterizes low intra-species (~2%) with simultaneously high interspecies variability, flanking by conservative domains and short length (up to 600 base pairs). Analysis of the ITS region (internal transcribed spacer) has proved to be effective in the identification of species within a number of genera: *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Ulocladium*, *Scopulariopsis* and *Mycelia sterilla*. There was a failure in the precise sequence analysis of closely related *Penicillium*

species (*Penicillium freii*/*P. polonicum*, *P. ochrochloron*/*P. pulvillorum*, *P. bialowieziense*/*P. brevicompactum*), but in such cases, the  $\beta$ -tubulin region was used effectively. The ITS region also demonstrated some limitations during the diagnostics of fungi from *Cladosporium* and *Alternaria* genera. Additionally, translation elongation factor 1- $\alpha$  was specific for *Cladosporium* and *Fusarium*, but not for *Alternaria* (K. Plewa, E. Lonc; University of Wrocław).

Yeasts and yeast-like fungi are important components of many ecosystems and possess economic, agricultural and medical importance through their considerable impact on fundamental soil processes, such as decomposition, aggregation, nutrient release and storage, as well as their interaction with vegetation and soil animals. The species composition of different localities is highly heterogeneous and the quantities of fungi range from hundreds to millions of cells per gram of soil. Up to 130 species have been reported from soils worldwide [23,24].

Fungi may become potential human and animal pathogens, especially for individuals with a depressed immune system. The presence of fungi in the environment, especially in soil, may be a factor favouring their spread into human ontocenoses. An evaluation of soil samples taken from child recreation sites in Łódź in autumn 2010 and spring 2011, revealed a variety of species: *Candida colliculosa*, *C. guilliermondii*, *C. humicola*, *C. inconspicua*, *C. lambica*, *C. lusitaniae*, *C. pelliculosa*, *C. tropicalis*, *Cryptococcus albidus*, *C. laurentii*, *C. neoformans*, *C. terreus*, *Kloeckera japonica*, *Geotrichum candidum*, *G. penicillatum*, *Rhodotorula rubra*, *R. glutinis*, *Saccharomyces cerevisiae*, *Sporobolomyces salmonicolor* and *Trichosporon cutaneum*. The most common source of *Cryptococcus* isolates were the soil of sandpits, school pitches and park playgrounds. Moreover, *Cryptococcus neoformans*, an etiological factor of cryptococcal meningitis, was also present in the sandpits of 3 kindergartens. The *Candida* fungi were isolated in soil samples from all unfenced park playgrounds and unfenced school pitches only in autumn. The number of colony forming units (cfu/1g of soil) of all isolates was greater in autumn than spring-time (A. Wójcik, J. Błaszowska, P. Kurnatowski, K. Szwab; Medical University of Łódź UM).

The characteristics of pathogenic fungi isolated from three natural ponds in the Białystok area was presented (B. Kiziewicz, Medical University of Bia-

lystok). In autumn 2011 and spring 2012, water samples were collected from three ponds within the town of Białystok: Fosa Pond, Dojlidy Pond and Komosa Lake. Water samples for examination were obtained 0.20 m under the surface of the water using Ruttner's apparatus (2.0 dcm<sup>3</sup> capacity). A bait method using seeds as bait was applied to isolate fungi. The samples were stored for approximately one month at a temperature of 18°C. The bait was successively observed under an optical microscope (100 and 400x magnification) every 3–5 days, starting from day 3 of the culture. The fungi were identified based on the following morphological features: the shape and size of the thallus, the shape of the sporangium and spores, the structure of the oogonium, gametangium and oospores. These microscopically classified mycelia were removed from the seeds and transferred to sterilized Petri dishes containing Sabouraud's medium or Potato Dextrose Agar (PDA). This study revealed the occurrence of 30 species from 5 orders: Chytridiomycetes (5), Zygomycetes (1), Oomycota (19) and anamorphic fungi (5). The most common species were *Aphanomyces laevis*, *Catenophlyctis variabilis* and *Saprolegnia ferax*. Three new species were discovered: *Achlya diffusa*, *Pythium gracile*, *Saprolegnia litoralis*. The following potential pathogenic fungi were determined: *Lagenidium giganteum*, *Catenophlyctis variabilis*, *Rhodotorula glutinis*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus candidus*, *Penicillium chrysogenum*. The isolation of anthropopathogenic fungi from water samples suggests that natural aquatic reservoirs are an important source of fungal infections. These species can be potentially applicable as an indicator of water contamination, and can be useful in sanitary-hygienic assessment.

The WHO defines zoonoses as diseases and infections that are naturally transmitted between vertebrate animals and humans. Protozoan and helminth zoonoses are of great importance in public health. In urban areas, the main source of many parasitoses is soil contaminated with feline or canine faeces. The most frequent intestinal parasites of dogs are helminths e.g. *Toxocara canis*, *Toxascaris leonina*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Trichuris vulpis* and *Dipylidium caninum*. Nematodes, like *Toxocara cati*, *Toxascaris leonina* and *Ancylostoma tubaeforme* are common parasites of the alimentary tracts of cats. Most of them can infect humans [25,26], but *T. canis* and *T. cati* have the greatest epidemiological importance [27]. The prevalence of

intestinal parasite infections in dogs and cats from the Lodz Shelter for Animals was assessed (K. Szwabe, J. Błaszowska; Medical University of Lodz). A total of 137 fecal samples from 87 dogs and 50 cats were examined by a sedimentation method using a Mini Parasep faecal parasite concentrator. The overall prevalence of intestinal parasites in dogs and cats were 35.8% and 58%, respectively. Five different parasite species were observed in stray dogs, and eight species were detected in samples from the cats. In dogs, the prevalence of parasites found were: *Toxocara canis* (18.1%), *Uncinaria stenocephala* (8.1%), *Trichuris vulpis* (5.8%), *Capillaria* spp. (2.3%) and *Isospora* spp. (1.2%). In cats, the following species were detected: *Toxocara cati* (32%), *Ancylostoma caninum/Uncinaria stenocephala* (2%), *Capillaria* spp. (2%), *Dipylidium caninum* (6%), *Taenia taeniaeformis* (2%), *Spirometra erinacei* (2%), *Isospora* spp. (16%) and *Giardia felis* (2%). *Toxocara* was the most frequently found worm genus in dogs as well cats. In 20% of canine samples mixed infections were found (*T. canis* and *Uncinaria stenocephala*; *Trichuris vulpis* and *Uncinaria stenocephala*; *T. canis*, *Uncinaria stenocephala* and *Capillaria* sp.). In the examined cat population, more than one parasite were observed in 8% of fecal samples (*T. cati* and *Isospora* sp., *Giardia felis* and *Isospora* sp.). The results obtained in this study are evidence that the greater part of animals adopted by the Lodz Shelter were infected with intestinal parasites. Parasite eggs, cysts or oocysts present in faeces can be transmitted to the soil and are a potential source of human infections.

Filariasis is a parasitic disease caused by thread-like nematodes, transmitted by blood-feeding arthropods: mainly black flies and mosquitos. The disease can be generally divided into three main types: lymphatic (caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, where worms are found in the lymph nodes, what can lead to elephantiasis), subcutaneous (caused by *Loa loa*, *Mansonella streptocera* and *Onchocerca volvulus*, occupying the subcutaneous layer of the skin, involving in river blindness) and serous cavity disease (caused by *Mansonella perstans* and *M. ozzardi* discovered within the abdomen with hydrocele). As international travel increases, there is a rising exposure to many pathogens not traditionally encountered in the resource-rich countries of the world. The GeoSentinel

Surveillance Network, a global network of medicine/travel clinics, was established in 1995 to detect morbidity trends among travellers. Filariasis comprised 0.62% (n = 271) of the 43 722 medical conditions reported to the GeoSentinel Network between 1995 and 2004. Immigrants from sub-Saharan Africa were most likely to have acquired a filarial infection than other travellers and long-term travel (greater than 1 month) was more likely to be associated with acquisition of filariasis than shorter-term travel [28].

A case was described of a 50-year-old man, who was admitted to the Department of Infectious Diseases in May 2008 with cellulitis, panniculitis and lymphangitis of the left crus with moderate fever. All symptoms had started in February 2008. The patient had been suffering from chronic headache, which had lasted for many years without any reaction towards non-steroidal anti-inflammatory drugs or carbamazepinum. He had travelled twice to India in the 1980s and Africa (Namibia) in 2005. The initial diagnosis was erysipelas, eosinophilia was not noticed and patient received the standard treatment. In June 2008, the patient was readmitted to the hospital due to renal and liver failure, bronchitis, left thigh elephantiasis and gonocoele. No fever, leukocytosis or eosinophilia were noticed. Thick and thin blood smears, haematoxylin stained, revealed the presence of microfilariae probably connected with *W. bancrofti* infection. The second case concerned a 58-year-old patient with generalized myalgia and arthralgia, weakness and enlargement of neck lymphatic nodes lasting or less than six months. This patient had been in Kenya, where he had gone on safari, for 14 days almost 7 years before. There were no changes in general and serological tests and as in the first case, blood smears revealed parasites. These presented cases underline the necessity of blood smear evaluation in patients with a suspicion of parasitic disease and no eosinophilia, even if it has been many years since they might have travelled to endemic parts of the world before the present symptoms occurred (Z. Deroń; Bieganski Voivodship Specialist Hospital in Lodz).

Malaria, a mosquito-borne infectious disease of humans and other animals caused by protozoan parasites of the genus *Plasmodium*, is still a major global problem, causing about 250 million clinical cases a year. It is responsible for nearly 800 000 deaths a year, almost all in people living in poverty with limited access to healthcare, but it also causes

avoidable deaths every year from imported malaria in non-endemic countries. Such a situation is strictly related to the increased rate of travel to endemic areas and a lack of knowledge about the risk of malaria, both by tourists and their physicians. This is the main reason why, in many European countries, there is no pre-travel advice and therefore no diagnosis and proper treatment upon return. The number of cases of imported falciparum malaria has increased over the past four years in the United Kingdom with 5774 reported cases between 2007 and 2011. Most travelers acquiring malaria are of African heritage visiting friends and relatives. In contrast, the risk of dying from malaria once acquired is highest in the elderly, tourists and those presenting in areas in which malaria is seldom seen [29].

Between January 1999 and September 2003, a total of 4 801 patients with travel-related malaria were reported within the TropNetEurop Network. *P. vivax* was present in almost 13% of cases, either as the sole pathogen or in mixed infections with other species, thus accounting for the second highest number of cases after *P. falciparum*. The reported proportion of *P. vivax* remained steady with 10.9% in 1999, 12.6% in 2000, 15.1% in 2001, 12.3% in 2002 and 12.9% in 2003. Of note, Germany (24.3%), Spain (15.5%) and the UK (12.0%) reported most cases, whereas reports from Switzerland (1.8%), Poland (1.6%), Finland (1.0%), Ireland (1.0%) and Portugal (0.3%) were scarce. According to diagnostic information, 557 (90.1%) of the 618 infections were confirmed, two (0.3%) were probable and eight (1.3%) were suspected. The remaining 51 (8.3%) could not be classified due to missing information on the underlying diagnostic procedure [30].

*Plasmodium vivax* infections are characterized by relapses of malaria arising from persistent liver stages of the parasite (hypnozoites) which can be prevented only by anti-malarials. A pertinent case is that of a 72-year-old patient, an internal medicine specialist, who was admitted to the Department of Infectious Diseases in 2004 due to moderate fever (Z. Deroń; Bieganski Voivodship Specialist Hospital in Lodz). He had a malaria in youth while working in Georgia. Initial diagnostic procedures confirmed only carries. The patient was given arechin, as if during a malaria attack, with transient improvement. Anti-*Plasmodium* antibodies were negative, but a basic screening test including evaluation of thick and thin blood smears,

which was the only chance to make a correct diagnosis, was not performed. After a consultation with the infectious disease specialist the patient was discharged from the hospital with antiprotozoal drugs eliminating the liver reservoir, but the treatment was delayed. After 4 months, the patient was readmitted to the hospital due to fever. Despite a confirmed plasmodium infection, he was not properly treated and serious complications, including heart failure and encephalopathy, occurred. In 2007, the patient experienced another relapse. Thick and thin blood smears once again confirmed recurrent malaria caused by *P. vivax*. Treatment with arechin interrupting the attacks, followed by primachin, proved to be effective. This case indicates the need for blood smear evaluation when malaria is suspected, followed by treatment of the attacks and eradication of the liver reservoir.

*Toxoplasma gondii* is a prevalent obligate intracellular parasite which has chronically infected more than a third of the world's population. More recently, it has been learned the *T. gondii* infection has the ability to modify host behaviour [31]. A correlation has been identified between latent toxoplasmosis and human behavioral changes such as slower reactions, lower rule consciousness and increased activity as well as deficits in learning capacity and memory. Studies show that these parasites may be a causative or contributory factor in such psychiatric disorders as depression, anxiety, schizophrenia, Parkinsons disease and epilepsy [32,33]. Behavioral changes in mice infected by *Toxoplasma gondii* were estimated (J. Gatkowska, M. Wiczorek, B. Dziadek, K. Dzitko, H. Długońska; University of Łódź). The experiments were performed on C57BL/6 inbred mice, which are innately highly susceptible to *T. gondii*. The intermediate virulent ME49 strain of *T. gondii* was used to induce experimental toxoplasmosis. Mice were infected by intraperitoneal administration of 20 cysts per mouse. The behavior of non-infected mice and mice infected acutely (3 weeks p.i.) and chronically (6 weeks p.i.) with *T. gondii* was quantified in an open field (OF) using a 59×59×30 cm box with its floor divided into center and peripheral parts illuminated by red light. Each mouse was placed in the center of the arena facing the wall, then videotaped for 5 min and scored with the EthoVision system. Three groups of behavioral parameters were measured: 1/ exploratory activity (rearing and climbing on the walls), 2/ spontaneous locomotor activity (entries to the central and peripheral parts of the OF arena),



and 3/ anxiety-like behavior (self-grooming). Infected mice showed significantly diminished exploratory activity described by climbing and rearing, reduced preference for the central, more exposed part of the OF arena and engaged in less grooming behavior compared to the uninfected controls. The locomotor activity of infected mice was most pronounced in the peripheral part of the open field especially during acute parasitosis. This study confirmed that the presence of *T. gondii* cysts in the brain caused behavioral changes in mice. The cysts were detected during late acute (3 weeks p.i.) and chronic (6 weeks p.i.) toxoplasmosis both in the hippocampus and the amygdala of infected mice. According to some investigators, these structures of the brain are involved in defense behaviors, such as conditioned or learned fear, and nonconditioned anxiety [34,35].

Infection with the protozoan *Toxoplasma gondii* causes serious public health problems and is of great economic importance worldwide. The standard therapy of toxoplasmosis is the combination of pyrimethamine plus sulfadiazine or clindamycine. However, this treatment is often associated with severe side effects including allergic reactions and bone marrow suppression. For this reason, there is an ongoing search to find new drugs to counter this form of parasitosis. Thiosemicarbazone and thiadiazole derivatives are of considerable interest because of their, among others, antiprotozoal, antibacterial and antitumor activities. The anti-*Toxoplasma* activity of newly synthesized thiosemicarbazides and thiadiazoles have been estimated (K. Dzitko, A. Ruszczak, A. Siwek, H. Długońska; University of Lodz, Medical University of Lublin). In preliminary studies, these compounds were tested for *in vitro* cytotoxicity, using mouse L929 fibroblasts and HeLa cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Tetrazolium salts are widely used as indicators of metabolic activity for both eukaryotic and prokaryotic cells. Live cells reduce the tetrazole ring in the MTT salt, forming a colored formazane which can be assessed spectrophotometrically. In the present study, the MTT test was used to assess the survival of extracellular tachyzoites in other concentrations of samples. In addition, [<sup>3</sup>H] uracil incorporation was used to evaluate the efficacy of new compounds against the proliferation of *T. gondii* in vitro in mouse L-929 fibroblasts. These results showed that the tested thiazolidinone and thiadiazole derivatives decreased proliferation of

*T. gondii* in L-929 cell line. The DL<sub>50</sub> values of the majority of tested compounds for intracellular tachyzoites were lower than the DL<sub>50</sub> of sulfadiazine (DL<sub>50</sub> > 500 µg/ml). These chemicals were not seen to have any influence on extracellular tachyzoites. Of the newly synthesized thiosemicarbazides and thiadiazoles, the highest anti-*Toxoplasma* effect was demonstrated by 4-(4-fluorophenyl)-1-(tiofen-2-yl)-carbonylthiosemicarbazide (DL<sub>50</sub>=33.17 µg/ml).

The detection of *Toxoplasma*-specific antibodies is the primary means of diagnosing infection with *Toxoplasma gondii*. Several tests to identify the presence of immunoglobulin IgG have been introduced to help discriminate between recently acquired and distant infection. *Toxoplasma* antibody detection tests are performed by a large number of laboratories with commercially available kits. A comparative evaluation of four *Toxoplasma*-specific IgG antibody tests, (three commercial and one not), and their significance in different clinical settings was conducted by E. Gołąb, G. Karczewski and M. Bakal-Nowak (NIZP-PZH, LUX MED; LIM Medical Centre, Warsaw). Seventy serum samples were examined to identify the presence of immunoglobulin IgG by the following four tests: 1/ chemiluminescent microparticle immunoassay (CMIA) – Toxo IgG, Architect System (Abbott Diagnostics Division), 2/ an enzyme-linked fluorescent assay (ELFA) (VIDAS Toxo IgG, bioMérieux), 3/electrochemiluminescence assay Cobas (Roche Diagnostics GmbH), 4/ indirect immunofluorescent-antibody assay - IFAT (National Institute of Public Health -National Institute of Hygiene, Poland). Antibody levels were evaluated by following the manufacturer's instructions where appropriate. In this study, an initial detection of IgG antibodies to *T. gondii* was performed with the CMIA – Toxo IgG, Architect System kit. Of 70 serum samples, 20 were negative, 49 samples were positive and one was a doubtfully positive result. All negative serum samples were confirmed by other applied tests (criteria for determining of negative results: for Architect Toxo IgG assay and IFA test – IgG level < 1.6 IU/ml, for Cobas < 1.0 IU/ml, Vidas < 4.0 IU /ml). The serum sample considered doubtfully positive by consensus was found to be indeterminate with the CMIA; this sample was also found to be doubtful by VIDAS, but positive by Cobas and IFAT. Of the 49 positive samples, no differences were found between the VIDAS and IFAT test results with regard to mean I.U. of IgG/ml of serum obtained, but these differences were obse-

rved in samples examined by the Cobas and Architect tests. The partial agreement of the results from the different commercial assays indicates that these tests are unreliable in diagnosing *T. gondii* infection when serum immunoglobulin IgG concentrations are low. It is worth emphasizing that serum IgG anti-*Toxoplasma gondii* antibody titres (expressed in I.U.) are dependent on the method of measurement used. Hence, only one test should be used to monitor *Toxoplasma*-specific IgG levels in the patient.

Leeches are ectoparasites on various vertebrates, including humans. After the leech attaches with its suckers to the host, the salivary glands secrete a number of compounds including an anesthetic, a vasodilator, a spreading factor and proteolytic inhibitors. A leech can suck and store blood up to ten times its body size. They can stay alive for about two years with blood stored in their crop. It has been shown that bacteria, viruses, and parasites from previous blood sources can survive within a leech for months, and may be transmitted to a human host. The ingested blood in the leech digestive tract could be re-injected into the host, along with the various pathogens, by regurgitation during the manipulation of leech removal. Leech bites have been associated with *Aeromonas veronii* biovar *sobria* infections, complicating up to 20% of medical leech therapies, but might also be involved in the pathogenesis of various tropical soil-related infections, such as *Chromobacterium violaceum* septicaemia and chromoblastomycosis [36]. Moreover, internal hirudinoses have been described in the literature. Sites of leech infestation include the nasal cavities, pharynx, larynx, respiratory tract, vagina, urinary bladder and rectum [37]. A rare case of an intraperitoneal hirudinosis in a 2-year-old girl has been described [38]; the leech entered her vagina and uterus, perforated the uterus and entered into the peritoneal cavity. The presence and survival of pathogens inside the gut of leeches has been studied [39]. HIV and hepatitis-B virus, bacteriophages and bacteria have been found to persist in large numbers for at least 6 months in the gut of experimentally infected leeches. Protozoan parasites such as *Toxoplasma gondii*, *Trypanosoma brucei brucei*, or *Plasmodium berghei* were even capable of reproducing inside the gut of the leech, and they survived about 5–6 weeks.

Leeches are currently in use in Medicine, especially in plastic and reconstructive surgery, wound and flap healing, in venous insufficiencies, and in the treatment of many disorders such as hemorrhoids and varicosity. The European Medical

Leech includes three species: *Hirudo medicinalis*, *H. verbana* and *H. troctina*. However, due to hirudotherapy complications such as infections by microorganisms, anaphylaxis, local allergic reactions, anemia and cellulitis can occur. A high incidence of *Aeromonas* infection after application of medicinal leeches, despite their external decontamination, necessitates an antibiotic prophylaxis. For this reason, an important clinical and biological aspect of medical leeches is the identity of the microbiota in the digestive tract and also on body surface of these animals. In a study conducted by Litwinowicz and Błaszowska (Medical University of Lodz), a mycological assessment was performed of the purity of the outer coating of medical leeches, as well as their jaws, other parts of their intestine, and also purity of water in which the leeches were kept. The study was performed on 20 *Hirudo verbana* obtained from the BIO-GEN company. Each leech was washed in 5 ml of Sabouraud's broth to rinse potential fungi out of the cover-muscular sack. Cultures were incubated at 37°C for 48–72h. The jaws, crop and intestine of leeches were incubated in Sabouraud's broth at 37°C for 48–72h. After centrifugation of 50 ml samples, 1ml of water were taken, inoculated on a Petri dish with solid Sabouraud's medium and then incubated for 48–72 h at 37°C. Mycological diagnostics were conducted using morphological and biochemical characteristics of the isolated fungi. Fungi were found in all samples from the cover-muscle sack, the jaws and samples of water. In total, 18 species of fungi from 8 genera were isolated in the study. No fungi were recorded in the intestine. The following species: *Candida krusei*, *Candida tropicalis*, *Rhodotorula rubra*, *Trichosporon asahii* and *Trichosporon asteroides* were isolated only from water samples. *C. albicans* was found in all tested materials. There was detected fungi classified as potential pathogens (BSL-2): *C. albicans*, *C. krusei*, *C. ciferrii*, *C. tropicalis*, *T. asahii* and *T. asteroides* as well as species with a lowered potential of pathogenicity (BSL-1), such as *C. guilliermondii*, *C. parapsilosis* or saprophytic fungi: *Lipomyces starkeyi*, *Rhodosporidium* sp., *Trichosporonoides* sp. Isolation of anthropathogenic fungi from the bodies and jaws of medical leeches, and the water they live in, suggests that they are an important source of fungal infections in patients exposed to hirudotherapy.

Humans live in constant association with the microorganisms present on the surfaces and in the

cavities of the human body, and even within cells. Over 500 species form the complex and dynamic communities which constitute the human microbiome (microbiota). The largest collection of microbes resides in the gut (10–100 trillion organisms). The gut microbiome genes and *Homo sapiens* genes together form the human „metagenome”, which can have a considerable impact on human physiology, nutrition and immunity, and may be a significant factor in the pathophysiology of many diseases. The intestinal microbial community is inherited from the mother. Knowledge of the functional genomics of microbial communities will allow the characterization of molecular signals and the mechanisms of the dialogue between microorganisms and their host. The creation of probiotic and prebiotic preparations specific for the aged population is one example of the medical/nutritional applications whose development has been spurred on by the growing body of evidence concerning the importance of gut microbiota homeostasis to the health of the host [40,41].

Establishment of the intestinal microbiota has been shown to be a progressive process. The major functions attributed to it begin to manifest at the end of the second year of life and comprise: nutrients absorption and food fermentation, stimulation of the host immune system and barrier effects against pathogens. Once climax composition is achieved near the end of adolescence, this ecosystem displays a high stability in healthy adults but recent studies indicated that modifications occur in the composition in elderly individuals [42].

It was revealed that bacteria of the two most important phylogenotypes of gastrointestinal tract (GI) microbiome, *Firmicutes* and *Bacteroides*, do not have their external reservoir. This observation proved the theory of vertical transmission of GI bacteria. Some studies suggest that there must be a pathway of transmitting signals from GI microbiome, through L-cells' receptors localized in colon secreting peptide hormones, to regions in the central nervous system responsible for metabolism's regulation.

The Human Microbiome Project (HMP), a United States National Institute of Health initiative, has been created in order to examine microbiome changes and its influence on human health. Understanding the nature of health can allow the clinical target of therapy to be more precisely defined. Many illnesses are ecological diseases

rather than the result of exposure to a rare pathogen. The knowledge of the microbiome enables an insight into areas of diet and body chemistry, and hence, into their associated fundamental metabolic pathways (A. Jaworski; University of Lodz).

In the available literature, there are several studies concerning treatment using fecal bacteriotherapy, a treatment first described in 1958 and now often referred to as fecal microbiome transplantation. During such a procedure, the bowel is cleaned with antibiotics and then infused with donor-processed fecal suspensions daily for 5 to 15 days. After 24 weeks, the donor flora is relatively stable in the microbiota of the feces. This method is suggested to be effective for new therapies in the treatment of colonic or metabolic disease. Other studies evaluated the treatment of *Clostridium difficile* diarrhea by fecal transplantation, resulting in therapeutic success after 14 days, which was then seen to last for almost 4 years. Microbiome transplantation method is still very controversial and not accepted by Food and Drug Administration (FDA) and other institutions responsible for introducing new medical procedures worldwide [43].

The occurrence of yeast-like fungi and yeasts in the oral cavity is regarded by some authors as a physiological state, and by others as a sign of the violation of immunological barriers. According to the literature, the prevalence of fungi in the oral cavity can vary between 10 and 96% [44]. The most commonly isolated fungi from the genus *Candida*, although in recent years, the frequency of the genus *Saccharomyces* has increased. One factor favoring the colonization of the mucous membranes by fungi and the development of mycosis is a high-carbohydrate diet. One reason for the greater consumption of monosaccharides is the significantly greater availability and popularity of various types of sweetened beverages. However, information about the connection between the consumption of such beverages and the development of fungi is scarce. One study investigated the correlation between frequent consumption of sweetened beverages and increased risk of fungal colonization in the oral cavity (K. Góralaska, P. Jabłoński, A. Klimczak, P. Rachubiński, D. Tyra, A. Kwapiszewska, A. Jagłowska, A. Borkowska, P. Kozarzewski; Student's Scientific Circles belonging to Chair of Biology and Medical Parasitology of Medical University of Lodz). Students completed an original questionnaire which

contained, among other things, information on the type, quality and quantity of beverages consumed. For mycological studies, oral washings were collected with liquid Sabouraud's medium, which were then incubated (temp. 37°, 24 h) and direct preparations were made. In the case of fungal isolation, mycological diagnostics were performed according to established procedures. The preliminary studies showed that fungi were present in the oral cavity of 52% of those examined, and the respondents declaring consumption of sweetened beverages were found to be associated with the increased prevalence of fungi (60–62.5%), compared to those who didn't. The study confirms that a diet high in sweetened beverages may favour the establishment of fungi.

In this article, a review of current problems of medical Parasitology and Mycology were presented, based on lectures and reports delivered during the 51st Clinical Day of Medical Parasitology (Lodz, 18 May 2012) as well as literature data.

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