Review articles

Clinical cases of parasitoses and fungal infections important from medical point of view

Błaszkowska Joanna[1], Katarzyna Góralska[2]

[1]Department of Diagnostics and Treatment of Parasitic Diseases and Mycoses, Medical University of Lodz, pl. Hallera 1, 90-647 Łódź, Poland
[2]Department of Biology and Medical Parasitology, Medical University of Lodz, pl. Hallera 1, 90-647 Łódź, Poland

Corresponding Author: Joanna Błaszkowska; e-mail: joanna.blaszkowska@umed.lodz.pl

ABSTRACT. Most important infectious diseases which pose a risk to human health and life are associated with parasites transmitted by a variety of arthropod vectors, or from animal to man. Some of these (malaria, toxoplasmosis, leishmaniosis, dirofilariosis, alveococcosis, cystic echinococcosis) still represent a serious public health problem in many regions in the world. This review describes the epidemiological and clinical aspects of important parasitoses and fungal infections from a medical point of view. It should be emphasized that the development of invasive disease depends on both host (susceptibility/resistance) and parasite factors (pathogenicity/virulence); an immunocompromised state can favour opportunistic parasitic infections: toxoplasmosis, cryptosporidiosis, giardiosis, cyclosporidiosis, blastocystosis and strongyloidosis. This article highlights the role of free-living amoebae in the pathogenesis and transmission of human diseases, the high pathogenicity of *Echinococcus multilocularis*, and the growing importance of ticks as a reservoir and vector for numerous dangerous pathogens (e.g., *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti*). It also discusses the diagnostic problems of toxoplasmosis including cross-reactions in serological tests and reviews the search for new drugs and vaccines against toxoplasmosis. Attention is increasingly paid to the role played by the human microbiome in maintaining homeostasis and in the development of fungal infections. This review also presents the most common human superficial fungal infections and the role of *Candida albicans* infection in the pathogenesis of irritable bowel syndrome.

Key words: parasitoses, zoonoses, opportunistic parasitoses, fungi, human health

As reported by Cox [1], humans are hosts to over 300 species of parasitic worms and over 70 species of protozoa. According to WHO data, in the last 10 years, more than 4.5 billion people in the world were infected with various parasites. Some parasite invasions may lead to serious and even life-threatening diseases. Of all parasitic diseases, malaria causes the most deaths. In 2015 alone, 214 million new cases of malaria were reported, of which approximately 438 thousand sufferers died, most of them in sub-Saharan Africa [2]. *Plasmodium falciparum* is responsible for 90% of the deaths of malaria sufferers. In addition, an important problem for public health is associated with Neglected Tropical Diseases (NTDs) for example, such parasitic diseases as schistosomosis, lymphatic filariosis, onchocercosis and dracunculosis. NTDs affect more than one billion people, one-sixth of the world's population, living largely on low incomes in the rural areas of tropical countries.

It should be noted that the majority of invasive disease are zoonoses [3]. Taylor et al. [4] report that, of the 1415 known aetiological agents of infectious and invasive diseases in humans, over 61% (868) are transmitted from animals. Although the largest number of zoonotic agents is bacteria and rickettsia (538), many others are fungi (307), helminths (287), viruses (217) and protozoa (66).

Some of the clinical, diagnostic and therapeutic problems associated with important parasitic and fungal infections were presented during the 55th

Clinical Day of Medical Parasitology (Lodz, 3th June 2016).

Tissue parasites located in the central nervous system are a challenge for both diagnosticians and clinicians. The presence of parasites in the brain leads to a range of neurological symptoms such as headaches, encephalitis and meningitis, seizures, and increased intracranial pressure, as observed in the course of cerebral malaria, neurotoxoplasmosis, *Entamoeba histolytica* amebosis, infection caused by genus *Naegleria* and *Acanthamoeba*, the invasion of nematodes (*Toxocara canis*, *Trichinella* spp., *Strongyloides stercoralis*), cerebral cysticercosis (*Taenia solium*) and cystic echinococcosis (hydatidosis) and alveolar echinococcosis (*Echinococcus multilocularis*).

Recent attention has focused on infections with free-living amoeba, which can be facultative human parasites. The microscopic amphizoic amoebae belonging to the genera *Naegleria*, *Acanthamoeba* and *Balamuthia* pose risk to human health and can even be life threatening. Although all invading protozoa can cause changes in the host CNS, their epidemiology and pathology, and the clinical features of the infections, differ significantly [5]. Infection by the thermophilic *Naegleria fowleri* is acquired by exposure to polluted warm water in ponds, lakes and swimming pools, with the portal of entry being the olfactory neuroepithelium. *N. fowleri* causes primary amoebic meningo-encephalitis (PAM) which has an acute course with 98% mortality after three to seven days of exposure. The pathological changes are haemorrhagic necrotizing meningoencephalitis, mainly at the base of the brain, brain-stem and cerebellum. Trophozoites can be seen within CNS lesions located mainly around blood vessels.

Balamuthia mandrillaris and several species of *Acanthamoeba* enter through the respiratory system or broken skin. They can invade the CNS by haematogenous dissemination causing granulomatous amoebic encephalitis (GAE), although this occurs more frequently in individuals with compromised immune systems [6]. Both, the cysts and trophozoites are found in tissue. GAE is characterized by an incubation period ranging from a few weeks to a few months, and often has a chronic course with a mortality of nearly 100%. It is usually seen in debilitated, malnourished individuals, in patients with AIDS or those undergoing immunosuppressive therapy often for organ transplants. It is well documented that several

species of *Acanthamoeba* can also produce *Acanthamoeba* keratitis (AK), mostly in wearers of contact lenses or in individuals with minor corneal damage [7]. Recently, it has been suggested that the amoeba may act as a vector for various pathogenic microorganisms, especially bacteria of the genus *Shigella*, *Salmonella*, *Legionella* and *Pseudomonas* which are often present in the trophozoite vacuoles.

Hydatid cysts of tapeworms from the genus *Echinococcus* may be localized in the brain and threaten human life. The etiological factor of alveococcosis (alveolar echinococcosis) is the larva of *E. multilocularis*, whose range is limited to the northern hemisphere. The development cycle of the tapeworm is connected with definitive and intermediate hosts. In Europe, the main definitive host is the red fox or raccoon dog in the sylvatic cycle, and the dog in the synanthropic cycle. The most common mode of transmission to humans is by the accidental consumption of water or food that has been contaminated by *E. multilocularis* eggs. Endemic regions of alveococcosis occur in the western Alaska, the north-western portion of Canada, the Central Asian Republics, northern Japan, and western China. In regions with large populations of arctic foxes, the prevalence of *E. multilocularis* varies from a few dozen to as high as 400 per 100,000 inhabitants [8]. The prevalence of *E. multilocularis* in Europe has gradually increased, over recent decades. By the end of the eighties, this species was found in five countries (Switzerland, Austria, France, Germany, Russia), and has since extended its range to an additional 11, including Poland.

In Poland, alveolar echinococcosis in humans is classified as an emerging disease, and the number of detected cases has increased significantly over the last two decades. A total of 121 cases of alveococcosis were diagnosed in the years 1990–2011 [9]. Due to the high mortality rate of patients (80%), *E. multilocularis* was considered the most dangerous parasite in Poland. This tapeworm, together with the protozoan *Plasmodium falciparum*, is regarded as category four: i.e. pathogens which threaten human life (Dz. U. of 2002. No. 212, item 1798; List of pathogenic organisms and their classification and the measures required for the different degrees of the tightness). Alveococcosis develops slowly, over a few to more than a dozen years of asymptomatic infection. It is a progressive disease characterized by a tumor-like growth with a poor prognosis in patients: If

untreated, it usually leads to cachexia and death.

In humans, alveolar echinococcosis occurs less frequently than hydatidosis caused by *E. granulosus*. In the years 2006–2015, a total of 375 cases of *E. granulosus* invasion were registered; annually, an average of 20 cases (E. Gołąb; PZH,Warszawa). Morbidity was observed throughout the country, but most often in the Podlasie and Warmia-Mazury provinces. The epidemiological surveillance in Poland registers cases of cystic echinococcosis and alveolar echinococcosis by the State Sanitary Inspectorate on the basis of data sent by doctors and medical laboratories. The data is published in aggregate form by the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH) in annual bulletins and as "Infectious diseases and poisoning in Poland" (GIS). Symptoms of echinococcosis caused by *E. granulosus* depend on the location of hydatid cyst (HC) in the human organs; they are most frequently found in the liver (70%) and lung (20%) than the brain. This parasitosis may occur as an asymptomatic infection until complications arise from the pressure of HC on the surrounding tissues/organs or the HCs rupture, with a possible ensuing anaphylactic reaction. The species group *E. granulosus* sensu lato is well known to have great genetic variability: *E. granulosus* sensu stricto (G1-3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6-7, G8, G10) [10,11]. The genotype G1 is responsible for the great majority of human cystic echinococcosis worldwide (88.44%), and is often associated with transmission via sheep as intermediate hosts, while genotype G6 is known to be responsible for 7.34% of infections worldwide. Clinical data shows that G1 is most frequently localized in the liver, G8 in the lungs and G6 in the brain [12].

Data from the National Institute of Public Health-National Institute of Hygiene shows that 32 cases of trichinellosis infection were registered in 2014, 28 in 2015, and four by mid-October 2016 [13]. Worldwide, 10 thousand cases of the disease are recorded annually. In some parts of the world, invasions by *Trichinella* sp. are relatively rare; for example, the first case in Korea was recorded in 1997 after eating raw badger meat [14]. In other areas mass infections have been observed: Pozio et al. [15] describe an epidemic in Italy during which 92 people were infected, with the source being raw horsemeat. The cause of infection is the consumption of raw or undercooked meat of various animals, usually pork and wild boar. A study in Lithuania of 120609 boars during the years 1976–2013 found 2.5% to be infected with *Trichinella* sp. *T. britovi* dominated (91.9% of positive samples) while the remainder comprised *T. native* (7.7%) and *T. spiralis* (1.9%). In carnivores, the frequency of invasion ranged from 17% in foxes to 69.6% in wolves, with *T. britovi* being most frequently isolated: 96% positive samples [16]. Similar studies in carnivores conducted at the Institute of Parasitology, Polish Academy of Sciences, found a 20% prevalence of *Trichinella* in foxes and 46% in raccoon dogs (A. Cybulska; IP PAS, Warszawa).

Invasion occurs as a result of a complex combination of risk factors, in which the immune status of the host and the intensity of human contact with the etiological factor of parasitosis is crucial. Impaired human immunity is associated with the easily development of opportunistic parasitic infections, such as toxoplasmosis, blastocystosis, cryptosporidiosis, giardiosis, cyclosporidiosis and strongyloidosis. Immune deficits and immunosuppression allow the invasive form of parasites to penetrate into the human organism and develop further. While opportunistic pathogens tend to induce acute diseases in immunodeficient hosts, they often lead to asymptomatic parasitoses in immunocompetent persons. The most common agents associated with immune deficiencies are HIV infections, autoimmune and chronic diseases, radio-, chemotherapy, use of immunosuppressants, antibiotics and steroids. An immunocompromised host faces the possibility of infection with a subliminal number of parasites (cryptosporidiosis), activation of latent invasion (toxoplasmosis, leishmaniosis), reinvasion or superinvasion (toxoplasmosis), co-invasion (giardiosis and cyclosporidiosis, malaria and ascariosis), and increasing autoinvasion (hymenolepidosis, strongyloidosis). In HIV patients experiencing severe immunosuppression (CD4+ <200/ml), simultaneous parasitoses including neurotoxoplasmosis and cyclosporosis often occur, which can lead to death if left untreated.

Intestinal protozoa that cause mild to self-limited disease in an immunocompetent host can cause prolonged, intrac, recurrent and severe diarrhoea in HIV patients, resulting in weight loss and cachexia. The opportunistic parasite *Blastocystis hominis* has for a long time been considered as commensal, and its etiological role in human parasitosis has been a subject of controversy [17]. Now, two criteria have

been defined for considering this protozoon as a pathogen: its presence in large numbers, i.e. greater than five per high power field; 40×objective, and the absence of other potential pathogens. There is considerable genetic heterogeneity within the species and currently, more than 13 subtypes (STs) have been identified in humans and animals (mammalian, avian, reptilian) based on characterization of the small-subunit ribosomal RNA (SSU rRNA). *Blastocystis* spp. isolated from humans belong to the same subtypes found in animals, suggesting that animals may be reservoirs and enable zoonotic transmission by the faecal-oral route. Nine of these subtypes, ST1-ST9, have been detected in humans, but ST1-ST4 represent 90% of the total cases of blastocystosis detected in humans. ST3 is believed to be the predominant subtype worldwide and is most commonly associated with chronic diarrhoea in humans [18]. Of the six different morphological forms of *B. hominis* (cyst, amoeboid, granular, avacuolar, vacuolar, multivacuolar), amoeboid forms are often detected in patients with symptomatic blastocystosis [19]. A variety of symptoms have been attributed to *Blastocystis* infection; the most common being diarrhoea and abdominal pain. Other nonspecific symptoms such as nausea, bloating, flatulence and anorexia, have also been reported.

In the global scale, the prevalence of *B. hominis* in humans varies from 0.5% to 30% in industrialized countries, and from 30% to 76% in developing countries [20]. It is frequently detectable (18.5–60%) in patients with irritable bowel syndrome (IBS). An association between *Blasto-cystis* infection and acute chronic digestive disorders such as IBS have also been suggested. It is more prevalent in immunodeficient patients than immunocompromised persons. The pathogenicity of this protozoan increases in organ transplant patients. In Poland, the prevalence of *B. hominis* is estimated to be 0–15.3% depending on the examined population. *B. hominis* invasion was detected in 4.8% of a group of 62 adult patients from the Institute of Gastroenterology, Hepatology and Oncology in Warsaw, with chronic or recurrent intestinal ailments and treated with steroid drugs, while no such invasion was found in a group of 42 immunocompromised children with diarrhoea, hospitalized in a district hospital in Otwock (M. Bednarska, R. Welc-Faleciak, A. Bajer, I. Jankowska, M. Wielopolska, B. Wolska-Kuśnierz, A. Pawelas; UW). Other study showed higher the prevalence of

B. hominis (15.3%) in Polish military personnel returning from peacekeeping missions in Iraq and Afghanistan [21].

Worldwide, about 12 million people are infected with a protozoan of the genus *Leishmania*, and every year 1.5 to two million new infections are identified [22]. The promastigote, the form invasive to humans, is carried by mosquitoes of the genera *Phlebotomus* and *Lutzomyia*. *Leishmania* occurs in tropical, subtropical, southern Europe, Asia and Central and South America. It is considered endemic in 88 countries, and causes the death of 70,000 to 80,000 people annually [22]. The disease occurs in three forms: cutaneous, mucocutaneous and visceral, which are present at different frequencies in different parts of the world [23–25]. While the visceral form is often fatal, muco-cutaneous leishmaniosis often develops in patients who have had a cutaneous form, up to 90% of healed patients [26]. Mucocutaneous infection affects the nasal and oral cavities, including the hard and soft palate, chicks mucosa and gums (J. Moroz, P. Kurnatowski; UM, Łódź). Risk groups include those infected with HIV, diabetes, and long-time smokers. Painful changes in the oral cavity are often observed, including ulceration and bleeding from the gums and palate. The literature describes also a few cases of *Candida* invasion as a co-infection [25]. Repeated relapses are observed in as many as 7% of patients, despite initially successful treatment [26,27]. Potential targets for new therapies include the vesicles of the exosomes in which the cells of *Leishmania* sp. secrete virulence factors (M. Doligalska, UW).

The survival and transmission of pathogenic protozoa depends on their ability to evade or overcome the host's innate and adaptive immune responses. The development of strategies that increase immunity against protozoan parasites can help prevent infection or the reactivation of latent invasion. Besides the application of vaccines, the possibility of administration of exogenous interleukins order to stimulate cellular and/or humoral responses has been considered. Rodent models of protozoan infections have been extensively used to reveal host-pathogen interactions and pathways of immune regulation during acute and chronic parasitoses. Babesiosis and malaria caused by an intra-erythrocytic protozoan have similar clinical signs, and pathological lesions include severe haemolytic anaemia, icterus, neurological signs, pulmonary oedema, circulatory collapse and

acute kidney damage [28]. Regulatory cytokines such as IL-10 and transforming growth factor (TGF)-β are important to suppress the pro-inflammatory response. Elimination of *Babesia microti* infection is mainly effected through the production of TNF-α, TNF-ß and IFN-α, which activate neutrophils and macrophages, leading to enhanced phagocytosis. During the acute phase of babesiosis, the innate response is regulated by inflammatory and type-1 cytokines while during the chronic phases, the Th-1 cells and antibodies controlling parasitemia remain at a low level. The regulatory cells or IL-4 and IL-10 secreted by Th-2 inhibit the activation of macrophages. It is documented that IL-10 and TGF-β inhibit the Th1-dependent immune response in infected animals [28]. Recently, an attempt was made to resume the acute phase of *B. microti* infection in mice by the administration of exogenous IL-10 or TGF-β (J. Szymczak; UW). It was observed that administered IL-10 results in a slight increase in parasitemia in mice at day 9 or 10 following *B. microti* infection in comparison with controls. Additionally, IL-10 and TGF-β do not influence the proliferation of CD4+ CD25+ FoxP3+ regulatory T cells in the spleen of infected animals, and reduce the production of IL-12. Other observations suggest that B cells and CD4+ T cells are especially involved in the regulation of the immune response in mice after primary infection with *B. microti*, through production of gamma interferon (IFN-γ) and antigen-specific IgM and IgG [29]. Understanding the immune response to invasion with *Babesia* parasites is important for creating an effective vaccine.

Early diagnosis is critical for effective therapy, and the correct interpretation of serological tests for toxoplasmosis is very important, especially in pregnant women [30]. Unfortunately IgM, IgA, or IgE *Toxoplasma* antibody test results cannot be used reliably discriminate between recently acquired and distant invasions. The occurrence of false positive results in terms of the presence of IgM antibodies may be related, e.g., in patients with the presence of rheumatoid factor. In addition, problems are associated with a lack of quality control, and the specificity/sensitivity of many commercial serologic kits. Moreover, the lack of reproducibility of results obtained using a variety of immunodiagnostic tests often leads to incorrect interpretation of results. Commonly-available tests tend to be based on native lysate of *T. gondii*

tachyzoites, which is known to be the most difficult to standardize polyvalent antigen. Frequent problems are also associated with borderline results, which require repeat tests and cross-reactions in serological diagnosis of toxoplasmosis. A recent case study indicates that infection with Epstein-Barr virus (EBV) may give false-positive results for anti-*Toxoplasma* IgM (J. Czyżewska, J. Matowicka-Karna, M. Alifier, H. Kemona; UM, Białystok). A 27-year-old pregnant woman hospitalized for abdominal pain and muscle weakness presented swollen lymph nodes in the neck, armpits and groin on physical examination. Based on the positive result of anti-*Toxoplasma* IgM (but IgG-), and negative results for HIV and CMV-antibodies, the patient was diagnosed with toxoplasmosis. She was treated with pyrimethamine and cotrimoxazole for 20 days, but miscarried during hospitalization. Broader serological testing performed during a subsequent hospitalisation prior to pregnancy two years later revealed a positive result for antibodies against EBV. This case indicates the need for tests to be extended from the basic TORCH panel to include other pathogens including EBV in cases there the woman is pregnant.

The importance of effective therapy for reducing the parasite reservoir and its associated negative health consequences was demonstrated by the recipient of the Nobel Prize for Medicine for 2015 years for the discovery of two antiparasitic drugs (H. Długońska, UŁ). The therapies have revolutio-nized the treatment of malaria and filariosis, which affect the inhabitants of 100 countries around the world. Irish-born William C. Campbell and Satoshi Omura from Japan shared the prize for research that led to the development of the drug ivermectin, which has dramatically reduced the incidence of river blindness and lymphatic filariosis, and has proven effective against other parasitic diseases. Chinese-born Tu Youyou achieved the other half of the prize for discovering the drug artemisinin, which has significantly reduced the mortality rates for patients infected with *Plasmodium*.

Studies based on a mouse model have shown that infection with intestinal nematode *Heligmo-somoides polygyrus* may reduce the severity of symptoms of experimental inflammation of the spinal cord and brain. In infected animals, an increase of the proportion of regulatory T cells, macrophages and PMN II neutrophils is observed, together with migration of leukocytes into the cerebrospinal fluid (K. Krawczak, K. Donskow-

Łysoniewska, M. Doligalska; UW). Positive results suggest that some of the parasitic infections may serve as an alternative form of therapy in certain disorders of the central nervous system.

The lack of 100% therapeutic efficacy, their numerous side effects, and the increasing problem of drug resistance associated with of the antiparasitic drugs has stimulated the search for new synthetic compounds which might be used in the therapy of parasitoses. The search for new drugs exhibiting high efficiency and high selectivity for *T. gondii* is a leading target for laboratories worldwide. Potential candidates include pyrimidine, salicylanilides, triclosan, artemisinin, oryzalin, fluoroquinolones, naphthoquinones, quinazoline, quinoline, bisphosphonates, purines, thiosemicarbazones, and thiazolidinediones. For the first time, activity of thiosemicarbazides against *T. gondii* was documented by Liesen et al. [31]. The tested compounds showed better LD50 values for both infected cells and intracellular parasites than the standard drugs sulfadiazine and hydroxyurea. Recently, a series of thiosemicarbazides were evaluated *in vitro* for inhibition of *T. gondii* proliferation and host cell cytotoxicity (K. Dzitko, A. Paneth, L. Węglińska, T. Plech, J. Gatkowska, B. Dziadek, A. Szpiek, B. Skalski, H. Długońska, UŁ; UM, Lublin). All studied compounds (3.91–125 µg/ml) displayed significant and reproducible antiparasitic effects at non-toxic concentrations toward the host cells; experimentally determined IC50 values were two-times to fifteen-times higher than those of sulfadiazine. The results of this study suggest that the inhibitory activity of examined thiosemicarbazides towards *T. gondii* proliferation is connected with the electronic structure of the molecule [32].

It should be noted that despite many decades of intense research, no vaccines are currently available to control any human parasitic disease. The most advanced malaria vaccine candidate is RTS,S/AS01, a recombinant vaccine against the pre-erythrocytic stage of *Plasmodium falciparum* [33]. This vaccine is being assessed in a phase 3 trial at 11 sites in seven countries in sub-Saharan Africa. More than 20 other vaccines against important parasitoses (e.g., schistosomosis, Chagas' disease, leishmaniosis, onchocercosis) are currently being evaluated in clinical trials or are in advanced preclinical development. No effective vaccine against human toxoplasmosis is ready for commercial use. At present, only one commercial animal vaccine

(Toxovax) based on a live, attenuated S48 strain has been licensed for use to avoid congenital infection in ewes.

Numerous vaccination trials against toxoplasmosis have been performed in animal models, including inactivated or attenuated vaccine, subunit vaccine, genetically-engineered vaccine and DNA vaccine [34]. The rhoptry proteins, ROP5 and ROP18, secreted by *T. gondii* tachyzoites during the invasion of the host cell have been considered as promising vaccine antigens. Preparations of recombinant ROP5 and ROP18 antigens, expressed in *Escherichia coli*, have been found to be recognized by specific antibodies produced during acute and chronic infections in mice [35]. Other proteins from tachyzoites (SAG, MIC, GRA), bradyzoites (MAG, BAG) and sporozoites (SPA) have also been used for immunizing mice. A recent study evaluated the vaccine potential of three trivalent antigen-cocktails composed of rROP2+rGRA4+rSAG1, rROP2+rROP4+rGRA4 and rROP2+rROP4+rSAG1 against chronic toxoplasmosis in BALB/c mice. Of the three, rROP2+rROP4+rGRA4 and rROP2+rROP4+ rSAG1 were found to be very effective in the development of high-level of protection against toxoplasmosis. The respective numbers of *T. gondii* tissue cysts in the brains of mice immunized with these proteins were reduced by 84% and 77%, compared to the control PBS-injected animals. Another recent study found a stronger reduction of *T. gondii* tissue cysts in the brains of males (72%) than females (58%) immunized with a tetravalent subunit vaccine (SAG1 +ROP2 +ROP4 +MAG1) against *T. gondii* (J. Gatkowska, M. Wieczorek, B. Dziadek, K. Dzitko, J. Dziadek, H. Długońska; UŁ). However, these vaccines were observed to have only partially protective effects. It is thought that the vaccines based on antigens expressed in the single development stage do not confer complete protective immunity against *T. gondii*; as its life cycle has three major infectious stages, i.e. tachyzoites, bradyzoites (tissue cyst) and sporozoites (oocyst), an effective vaccine against *T. gondii* invasion should contain highly-immunogenic antigens derived from each stage.

From an epidemiological point of view, parasitic and fungal invasions which spread directly through a hospital environment represent an important public health problem. In this specific closed environment, cases of nosocomial infection usually affect immunocompromised patients and whose

with extensive surgical wounds, as well as those treated in the absence of hygiene. Invasions by pathogens have most commonly been described among patients undergoing chemotherapy and long-term treatment with steroids or antibiotics, and their transmission occurs by direct contact with an infected person (patient-patient, patient-medical personnel, patient-medical staff-patient) or through contaminated water, food, or material and medical equipment. The potential risks of infections associated with leech therapy are generally underestimated. Hirudotherapy is often used successfully in modern medicine, especially in plastic and reconstructive surgery [36]. Due to the presence of symbiotic bacteria of the genus *Aeromonas* in their gastrointestinal tract, and the possibility of microbiological contamination during breeding even under laboratory conditions, leeches should be considered as a reservoir of potentially pathogenic microorganisms. Transmission to the host may occur through various routes, including local contact between the patient and the surface of the body of the animal and its jaws, the introduction of microorganisms into the wound during blood collection, or re-injection into the host while removing the leeches. It has been confirmed that patient safety and achieving the desired therapeutic effect depend mainly on the microbiologic purity of the animals used. Finding an effective method of eliminating the potentially pathogenic microbiota of the leeches used in hirudotherapy could protect the patient from infection-related complications and consequences of their treatment, as well as from the necessity of providing prophylactic antibiotic therapy prior to applying the leeches. Recently, new methods have been developed to eliminate bacteria and fungi from the gastrointestinal tract and the body surface of the leech. Feeding leeches of the genus *Aeromonas* with a small portion of sterile blood supplemented with ciprofloxacin (20μg/mL) or cefotaxime (50μg/mL) prevents bacterial infection for up to 14 days for patients undergoing hirudotherapy [37]. The most effective elimination of fungi colonizing the jaw and the body surface of leeches was obtained for clotrimazole (20mg/L), which ensures the safety of the patient from fungal transmission up to 12 days (Litwinowicz; UM, Łódź). These described methods can be successfully used instead of antibiotic prophylaxis, which are currently prescribed as mandatory before and during hirudotherapy.

Over the past several years, scientists have been studying the interaction between the human body and the microorganisms inhabiting it [38]. The Human Microbiome Project Consortium is working on cataloging species occurring on the skin and mucous membranes, and within human organ ontocenoses, and attempting to determine their impact on the functioning of the host organism. As most publications in this area concern bacteria, which represent the largest group of commensals, other microbiota components are omitted, such as the fungi forming a kind of mycobiome within the human body (B. Dworecka-Kaszak; SGGW, Warszawa). Gouba and Drancourt [39] note that 390 species of fungi can occur on the skin and mucous membranes, and 335 in the gastrointestinal tract. The most common genera include *Candida*, identified in 75% of cases, *Cladosporium* (65%), *Aureobasidium* and *Saccharomycetes* (50%), *Aspergillus* (35%), *Fusarium* (30%) and *Cryptococcus* (20%). Particular areas of the human body vary in biodiversity: while 40–80 taxonomic units are found on the skin, 75–88 are found in the mouth, 50–118 in the gastrointestinal tract and 11–20 in the vagina [39–41]. The best known mycobiome is that of the gastrointestinal tract, and initial studies have found differences between mycobiota of healthy subjects and hospitalized patients. Forty-two species were isolated from patients with inflammatory bowel disease, 29 from individuals with HBV, and 11 from HIV-infected patients. Thirty-three species were found to be characteristic only for healthy subjects, 27 only for patients with inflammatory bowel disease, 17 for HPV infected patients, 16 for people with obesity and 10 for sufferers of anorexia [39]. Underhill and Illiev [40] suggest that in healthy persons, fungi present in the intestine, especially of the genus *Candida*, are metabolically adapted to commensal lifestyles and are incapable of inducing infection. Another situation is observed in patients with gastrointestinal disease. Fungi modulate the immune system through release of the cell wall components mannan, glucan and chitin, and it appears that their activity may be species-specific. In cases of Crohn's disease or inflammation of the intestinal mucosa, the presence of fungi increases inflammation by stimulating the function of the phagocytic cells [41]. In irritable bowel syndrome there was a positive correlation between the severity of symptoms and the presence of IgG and IgG4 antibodies against *Candida albicans* [42]. The clinical symptoms associated with infection with *C. albicans* are usually non-specific, such as nausea,

diarrhea, vomiting, or gastrointestinal bleeding (M. Gackowska, R. Wesołowski, K. Szewczyk-Golec, C. Mila-Kierzenkowska, J. Paprocki; CM, Bydgoszcz; UMK, Toruń). Colonization with fungi of the genus *Candida* in the course of inflammatory bowel disease and candidosis of the gastrointestinal tract are associated with elevated levels of pro-inflammatory IL-17 [43]. The duration of the disease is positively correlated with a greater likelihood of developing fungal infection as an additional factor of inflammation. Tests on an animal model have indicated that the presence of fungi in the course of ulcerative colitis causes an increase in the level of IL-1β and TNF-α, and slows down the healing process [44]. The incidence of *Candida* sp. in the intestine has been found to be similar in patients with Crohn's disease and their healthy relatives. This may suggest a form of sub-clinical inflammation in patients showing no symptoms. Inflammatory bowel disease affects the imbalance in the number and variety of microbiota of the gastrointestinal tract and supports the growth of fungi, while fungal growth leads to greater inflammation [42]. Zwolińska-Wcisło et al. [44] suggest that the use of probiotics in treatment simultaneously with antifungal drugs has a beneficial effect on treatment efficacy. Hanna and Cruz [45] describe a case of mastitis in a woman breastfeeding a baby with thrush, in which the applied antimycotics did not inhibit the symptoms, and improvement was achieved after the introduction of probiotics into the diet. Positive results were also observed in double-blinded clinical trial on women with recurrent vulvovaginal candidosis. Addition probiotics during routine treatment reduced the severity of symptoms and decrease the time of therapy [46].

Superficial fungal infections concern 20–25% of the human population [47–48]. Over several years of studies at the Medical University of Gdansk, covering almost 25,000 patients, it was found 25.5% rate of infection. Where 49.7% of all infections were caused by dermatophytes, 45.1% by yeast and yeast-like fungi, and 5% by molds. The most prevalent species were found to be *Trichophyton rubrum* and *Candida albicans* (A. Petranyuk, B. Bykowska, R. Nowicki; GUM). *T. rubrum* is considered to be the most common ethiological factor of dermatomycoses worldwide [48,49]. Despite this, there are noticeable differences in the prevalence of different species in different regions. In studies conducted among children in Nigeria, the incidence of infections was 35% and the most common species was *Microsporum audouinii*, representing 28% of the isolates [50]. The authors suggest that differences in the prevalence of fungi may be related to climate and environmental factors in different regions of the world.

Another recent threat to human health is the occurrence of *Anaplasma phagocytophilum* infection. Since the first known human infection in 1994, the rate of infection in the United States is 1.6 per million inhabitants [51]. A typical carrier of the bacteria of the genus *Anaplasma* is *Ixodes scapularis*, which is also a vector of *Borrelia burgdorferi* sensu lato and protozoa of genus *Babesia*. Other species of the genus *Ixodes*, i.e. *I. pacificus* and *I. ricinus*, may also participate in transmission [52]. In addition, *Dermacentor reticulatus*, which is involved in the spread of *Rickettsia helvetica*, is also a vector of *Anaplasma phagocytophilum* (J. Liberska, M. Dabert; UAM, Poznań). *A. phagocytophilum* causes human granulocytic anaplasmosis, of a different forms ranging from asymptomatic infection to severe multiorgan invasion. The most common symptoms can include fever, myalgia, headache, cough, nausea and vomiting. During the acute phase of the disease, characteristic morula can be seen in neutrophils in a microscope image of peripheral blood. Weil et al. [52] analyzed 33 cases of infections that occurred in a period of nine months in the area of Massachusetts: 42% of patients required hospitalization, only half of the patients were asked in an interview about tick bites in recent times, and only 11 of these confirmed this was the case. Morula forms were found in granulocytes in only five cases, which suggests that a blood smear is not a sufficiently sensitive method for the detection of these bacteria. Often identification must be conducted with molecular methods. The first case of human granulocytic anaplasmosis in Canada was recognized in a 82-year-old man. The tick, identified as *I. scapularis*, was removed in hospital. Interestingly, both the microscopic examination of the blood and the molecular techniques confirmed the presence of *A. phagocytophilum* in the patient, but the body of the tick was found to be negative for pathogens [53].

The best-known tick-borne pathogens are bacteria of the genus *Borrelia*, which are Gram-negative motile spirochetes. Within the genus, two physiological groups can be distinguished. The first causes Lyme disease, a typical example being

Borrelia burgdorferi, and ticks of the genus *Ixodes* are involved in the transmission. The second group is the aetiological agent of relapsing fever, for example *Borrelia miyamotoi*, and the vectors include *Ornithodoros* and *Argas* genera [54]. In the course of Lyme disease in approximately 70% of patients, the first symptom is erythema migrans, which occurs within one to three weeks following the tick bite. According to the recommendations of the Polish Society of Epidemiologists and Infectious Disease Physicians, other basic diagnostic criteria are borrelial lymphocytoma, actrodermatitis chronica atrophicans, Lyme arthritis, Lyme carditis and neuroborreliosis [55]. The literature describes few cases of typical erythema which occur despite the tick bite not being observed by the patient [56]. Tick-borne relapsing fever is characterized by fever and nonspecific symptoms, which may include vomiting, nausea, diarrhea, involvement of the central nervous system, and respiratory disorders. Adeolu and Gupta [54] conducted a thorough phylogenetic analysis within the genus *Borrelia*. They identified two clades based on an analysis of a sequence of 25 highly conserved proteins, the 16S rRNA units, conserved signature indels and conserved signature proteins: one clade responsible for Lyme disease, and the other inducing relapsing fever. DNA hybridization revealed a similarity of 91.33–99.34% between the species that cause Lyme disease and 82.51–99.34% between factors of relapsing fever. In contrast, the similarity between the two groups was 73.03–74.85%. Adeolu and Gupta [54] thus propose a separation into the genus *Borreliella*, comprising species causing Lyme disease, and *Borrelia,* comprising the species responsible for relapsing fever.

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