Annals of Parasitology 2015, 61(4), 207–220
doi: 10.17420/ap6104.10

Review articles

Parasites and fungi as risk factors for human and animal health

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ABSTRACT. Recent literature data suggests that parasitic and fungal diseases, which pose a threat to both human and animal health, remain a clinical, diagnostic and therapeutic problem. Attention is increasingly paid to the role played by natural microbiota in maintaining homeostasis in humans. A particular emphasis is placed on the possibility of manipulating the human microbiota (permanent, transient, pathogenic) and macrobiota (e.g., *Trichuris suis*) to support the treatment of selected diseases such as Crohn's disease, obesity, diabetes and cancer. Emphasis is placed on important medical species whose infections not only impair health but can also be life threatening, such as *Plasmodium falciparum*, *Echinococcus multilocularis* and *Baylisascaris procyonis*, which expand into areas which have so far been uninhabited. This article also presents the epidemiology, diagnosis and treatment of opportunistic parasitoses imported from the tropics, which spread across large groups of people through human-to-human transmission (*Enterobius vermicularis*, *Sarcoptes scabiei*). It also discusses the problem of environmentally-conditioned parasitoses, particularly their etiological factors associated with food contaminated with invasive forms (*Trichinella* sp., *Toxoplasma gondii*). The analysis also concerns the presence of developmental forms of geohelminths (*Toxocara* sp.) and ectoparasites (ticks), which are vectors of serious human diseases (Lyme borreliosis, anaplasmosis, babesiosis), in the environment. Mycological topics contains rare cases of mycoses environmentally conditioned (CNS aspergillosis) and transmissions of these pathogens in a population of hospitalized individuals, as well as seeking new methods used to treat mycoses.

Key words: parasites, fungi, natural microbiota, human health

The human environment is constantly changing, and this change can affect human health to varying degrees. The World Health Organization (WHO) defines health as a state of "complete physical, mental and social well-being" and not just "the absence of disease or infirmity". Every area of life can significantly contribute to the maintenance or loss of this status. All biological agents, be they viruses, bacteria, fungi or parasites, play important roles in homeostasis disturbances. Some of the issues related to parasites and fungi, which are risk factors for human and animal health were presented during the 54th Clinical Day of Medical Parasitology (Lodz, 15th May 2015).

In recent years, the balance between man and the environment has been significantly disturbed by profound changes in the environment and anthroposphere. In many regions of the world these changes have resulted in sudden outbreaks of dangerous diseases, such as the avian influenza virus epidemic of 2003, which resulted in dozens of human deaths, and the Creutzfeld-Jakob disease outbreak which in the period from 1996 to 2011 led to the deaths of 175 people in the UK and 49 in other countries. The WHO include bird flu and Creutzfeld-Jakob disease in a group of over 200 diseases known as zoonoses and communicable diseases common to man and animals.

A further epidemiological concern is the recent Ebola virus epidemic in West Africa (Guinea, Liberia, Nigeria, Senegal, Sierra Leone). Although the virus was first detected in 1976, so far there has been no of so many cases of the disease. The 2014 outbreak was the largest yet, with individual cases still being confirmed. During this epidemic, the first contagion was recorded in December 2013 in Guinea, and the number of confirmed patients had increased to over 4,500 within nine months [1]. This number had risen to 15,212 by November 2015, with the number of deaths being 11,299 [2]. This epidemic was notable for the fact that it was the first to include reported cases of infections outside Africa: cases were recorded in Italy, the UK, the USA and Spain. The spread of the virus to other continents represents an enormous challenge not just for Medicine and Pharmacy, but also for Biology and Economics (J. Gatkowska – University of Lodz).

Any imbalance in the body causes changes in the diversity and abundance of microorganisms, and conversely, any change in the microbial population affects the health of the human host. Hence, it is possible to infer the state of the body on the basis of commensals or pathogens colonizing it. For instance, the composition of the microbiota of the gastrointestinal tract in children with autism is different from that of healthy children, in that the number of bacilli of genus *Lactobacillus* is twice that found in non-autistic children, *Candida* sp. are often diagnosed, and fewer streptococci and gram-negative bacilli are found [3]. In addition, children with autism are more likely to have toxin-producing, anaerobic bacilli of *Clostridium histolyticum* present in the gastrointestinal tract [3]. Children with type 1 diabetes are more likely to display lower numbers of lactobacilli and greater numbers of anaerobic *Clostridium* genus than healthy children [4]. The gut microbiota of patients with colorectal cancer tend to contain higher numbers of *Fusobacterium nucleatum*, the bacteria responsible for dental plaque formation [5].

Since 2007, there has been intensive study on understanding the structure and role of human microbiota in maintaining the stability of ontocenosis. Since most of the species that inhabit the human body still cannot be cultured, the genetic material of gut bacteria is undergoing analysis by the Human Microbiome Project [6]. The human body contains ten times more microbial cells than its own, while the number of bacterial genes is 50–100 times greater than the number of human genes (A. Jaworski – University of Social Sciences, Lodz). Specific microbiota have already been observed in the fetus and are very similar to the oral ontocenosis of the mother [7]. In addition, a number of dependencies have been found between the functioning of the central nervous system and the biodiversity of the intestinal microbiota, known as the brain-gut-microbiota axis. Significant proportions of patients suffering from schizophrenia have been found to have changes within the gastrointestinal tract: 50% with gastritis, 88% with small intestinal inflammation and 92% with colitis [8]. Intestinal bacteria can stimulate the production of such neurotransmitters as serotonin, melatonin, acetylocholine and γ-aminobutrytic acid, as well as secrete them independently, thus affecting the functioning of the nervous system [9,10].

The human body is inhabited by numerous commensal microorganisms that contribute significantly to the maintenance of equilibrium [11]. A range of disorders exist, and the natural biodiversity of microbiota may contribute to the occurrence of many infectious diseases and disorders which for many years were not associated with microorganisms. Teenage acne frequently occurs as a result of the development of anaerobic bacteria *Propionibacterium acnes*, not only of hormonal changes [12]. The antibiotics and retinoids used in the treatment of acne have a number of side effects, and natural remedies, unfortunately, hinder the course of therapy as they are of questionable antibacterial efficacy. Plant compounds with activity against *P. acnes* are undergoing investigation as complementary methods to treat acne (P. Rachubiński – Student Research Society, Medical University of Lodz). Unfortunately, the effect of treatment on the diversity and functioning of the natural microbiota remains unknown.

An increasing number of studies suggest that treatments based on human macro- and microbiota may be possible in the near future (M. Doligalska – Warsaw University, Warsaw). The use of probiotic strains to assist the action of the digestive system and the prevention of fungal infections in the course of long-term treatment with antibiotics has been known for many years. *Lactobacillus rhamnosus* also inhibits anxiety and depression by regulating the expression of γ-aminobutrytic acid and corticosterone receptors in the central nervous system [13], while *Lactobacillus plantarum* may inhibit the development of cardiovascular disease through assimilation of cholesterol [5]. Recent studies suggest that other groups of microorganisms may also be used as a form of therapy. Some strains of *Escherichia coli* are known to produce colicyne, which inhibits the proliferation of tumor cells [14].

Studies on laboratory animals have demonstrated that the vesicular stomatitis virus selectively destroys tumor cells by inhibiting metastasis to the central nervous system [15]. Work is also underway on the use of „transplant" intestinal bacteria in the treatment of obesity, with positive results being observed in animal models [16]. Cirimotich et al. [17] suggest that *Enterobacter* sp. in the gut of mosquitoes produce free radicals which are lethal for *Plasmodium*. Increasing the number of these bacteria in the *Anopheles* populations can be an excellent way to reduce the spread of malaria in humans. Some researchers have also indicated the possibility of use in the treatment of some species of helminths, e.g., *Trichuris suis* in Crohn's disease or the free-living nematode in colitis [18,19].

Disturbances of the immune mechanisms often result in the development of invasive fungal infections. Oncological patients, particularly those undergoing chemotherapy and radiotherapy, recipients of bone marrow, lung, kidney or liver transplants, asthma patients undergoing steroid treatment or patients infected by HIV are all at increased risk of infection by molds [20,21]. Immunosuppressed patients are also at a significantly increased risk of developing invasive exogenous mold infections, and the presence of *Aspergillus* in the central nervous system is potentially life threatening. In one case of invasive aspergillosis, manifested by the presence of multiple tumors, the etiological agent was identified as *Aspergillus fumigatus* on the basis of molecular studies (Ł. Pielok – Medical University, Poznan). Numerous studies indicate that the diagnosis of invasive aspergillosis poses many difficulties. The symptoms are often non-specific, such as weakness, headache, elevated temperature or confusion, and they may also indicate the presence of non-infectious agents or a neurological disorder, and a CT image may indicate a cyst or tumors [22–24]. In many cases, it is not possible to detect the fungus by standard culture methods and molecular examinations are needed to detect the genetic material of the pathogen.

A study conducted at the Medical University of Lodz revealed that 82.9% of patients on intensive care units are colonized by fungi, and the dominant species is *Candida albicans* (61.7%). The isolates displayed a range of biochemical profiles and various sensitivities to commonly-used drugs (B. Modrzewska, K. Khalid, A.J. Kurnatowska). Changes in the oral mucosa, such as inflammation of the tongue, inflammation of the prosthetic or leukoplakia, are also conducive to fungal colonization, with fungi being isolated from such patients with a frequency of 81–96% [25]. The prevalence of fungi in individuals not belonging to risk groups is slightly lower, about 68%, although *C. albicans* still predominates with frequencies of up to 79% [26]. However, Moqbil and Kurnatowski [27] note that the frequency of this species decreases in the course of anti-cancer radiotherapy in favor of species originally resistant to fluconazole, such as *Candida glabrata* or *C. guilliermondii*.

Unfortunately, the reservoir of fungi can be both natural and anthropogenic environment. A mycological analysis of the soil samples from playgrounds, sports fields and parks in the Lodz area found molds to be present in all samples, while yeasts, yeast-like fungi and dermatophytes were rarely recorded (K. Góralska, P. Kurnatowski, J. Błaszkowska, A. Wójcik – Medical University, Lodz). Similar results have been obtained by researchers around the world. Areas belonging to hospitals or schools commonly reveal fungi of the genera *Aspergillus*, *Fusarium*, *Chrysosporium*, *Paecilomyces* and *Penicillium* [28–30]. All of these fungi have been isolated from patients with invasive fungal infections [31–33].

Special hazards are associated with the presence of fungi with a defined class of biosafety (BSL). Góralska et al. [34] report that nearly 25% of fungi were classified as BSL 2 (causing opportunistic infections and superficial), and over half as BSL 1 (causing mild conicydal infections). The most common dermatophyte species found in the soil were *Microsporum gypseum* and *Trichophyton terrestre*. However, samples of animal hair and skin scrapings originating from a fox farm revealed the presence of *Trichophyton mentagrophytes*. The tested animals showed no lesions (I. Dąbrowska – University of Life Sciences, Warsaw). Contact with the soil may favor the occurrence of superficial mycoses in farm animals and pets, even in immunocompetent individuals.

Trichophyton rubrum is the most commonly found dermatophyte within the human population, causing skin lesions and onychomycosis [35,36]. Onychomycosis affects 4.3–8.9% of the populations of Europe and North America [37]. However, onychomycosis was recorded in 43% of nursing home residents examined in one study, including 53% common dermatophyte infection, while it was impossible to determine the etiologic agent in as many as 36% of cases (P. Krzyściak – Jagiellonian University, Cracow).

Dermatophyte infection results in the nail plate becoming brittle and bold, with noticeable white spots and loss of transparency. These changes may be accompanied by pain, redness and swelling of the nail shafts, as well as secondary bacterial and yeast infections [38]. Unfortunately, due to the low permeability of drugs through the nail plate, treatment is often not very effective and is of a short-term nature [39]. In recent years, laser therapy has become increasingly popular (A. Erkiert-Polguj, B. Algiert, H. Rotsztein – Medical University, Lodz). In photodynamic therapy, healing was achieved in 43.3% of patients, but relapses of infection were observed in 26.6% of tested patients after a year [40]. The use of neodymium-yago laser directly to the culture of *Trichophyton rubrum* greatly limited the development of hyphae [41]. *In vivo* studies have demonstrated improvement in 81% of patients, and complete eradication of dermatophyte in 51% [42].

Opportunistic infections occur more frequently and are more severe in individuals with weakened immune systems. Among the intestinal protozoa, *Cryptosporidium* spp., *Giardia intestinalis*, microsporidia *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are prevalent in people with congenital or acquired immunodeficiency, including patients with AIDS [43]**.** These protozoal parasites can cause prolonged, recurrent and severe diarrhea, leading to weight loss and cachexia, and infection can even be life threatening. Microsporidia, like *Cryptosporidium* spp. and *Giardia intestinalis*, have been qualified by the National Institutes of Health (USA) and the Center for Disease Control and Prevention (CDC) as category B biological agents: as factors that could cause waterborne epidemics. Additionally, giardiosis and cryptosporidiosis belong to a group of neglected diseases.

A recent study has assessed the risk of opportunistic infections caused by parasites among patients in Poland (M. Bednarska, I. Jankowska, K. Piwczyńska, B. Wolska-Kuśnierz, A. Pawełas, M. Wielopolska, A. Bajer – Warsaw University, Warsaw). Among groups of patients with various immune statuses and recurrent and/or chronic diarrhea, intestinal protozoan infections were detected in 22/385 (5.7%) persons. Patients with compromised immune systems were infected by *C. parvum* and *C. hominis*, *C. meleagridis*, *C. felis*, *Cy. cayetanensi*s, *G. intestinalis*, *E. bieneusi* and *E. intestinalis*, while *G. intestinalis* and *Cy. cayetanensis* occurred in immunocompetent persons. The results of this study show that an important factor for the occurrence of intestinal infections is immune status: 86% of infected individuals (19/22) had immune deficiencies. The patients receiving immunosuppressive drugs before or after transplantation of organs demonstrate a higher risk of microsporidia infection while cryptosporidiosis develops most often in people with severe immunodeficiency. The recent study demonstrates the first case in Poland of *Enterocytozoon bieneusi* infection in a liver transplant patient [44]. Clinical observations confirm that in immunocompromised patients, the intestinal opportunistic parasites play a major role in causing chronic diarrhea.

Another species of cosmopolitan protozoa occurring in the human large intestine and now being regarded as an opportunistic parasite is *Blastocystis hominis.* This protozoan has several morphological forms (vacuolar, granular, amoeboid, cyst) with different cell sizes (2–200 µm), which makes it difficult to diagnose. For a long time, this species has been considered as commensal because even an intense invasion may be asymptomatic in immunocompetent persons. However, two criteria have been defined for considering *B. hominis* as a pathogen: 1) presence in large numbers (greater than 5 per high power field), and 2) diagnosis in the absence of other potential pathogens [45,46]. The prevalence of this protozoan ranges from 0.4 (western Australia) to 45% (Thailand) depending the climate and social conditions [47]. *B. hominis* infection is more common in developing countries in the tropical and subtropical regions. Additionally, *Blastocystis* spp. are commonly found in stools from humans and numerous animals, which suggests that it has zoonotic potential. However, there is considerable genetic heterogeneity within *Blastocystis* organisms, and currently up to 13 subtypes (STs) have been identified in humans and animals (mammalian, avian, and reptilian) based on characterization of the small-subunit ribosomal RNA (SSU rRNA) gene. Nine of these subtypes, ST1-ST9, have been detected in humans. ST3 is the predominant subtype distributed worldwide and is most commonly associated with chronic diarrhea in humans [48]. However, asymptomatic infection of protozoan parasites is also common in developed countries, and several subtypes have been isolated from healthy individuals without diarrhea (0.5% to

16.5%) [45]. Recently, the prevalence of *B. hominis* in the human population of Olsztyn and its surrounding area was estimated on the basis of ELISA enzyme immunoassay, and DNA analysis (M. Lepczyńska, E. Dzika, J. Korycińska, K. Kubiak – University of Warmia and Mazury, Olsztyn). The ELISA test identified *B. hominis* in 26% of subjects, and PCR assay in 12%. Women were more frequently infected (81% of the entire population) than men, and a higher prevalence of *Blastocystis* spp. was observed among rural residents (68% of the examined population).

Persons traveling to the tropics and subtropics may be at increased risk of coccidian infections. Enteric pathogens, such as *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli* and *Sarcocystis hominis*, are classified as unicellular human coccidians, which primarily infect the intestinal epithelium [49]. The key factor contributing to infection is the direct person-to-person (fecal-oral) transmission of mature oocysts, however, the oocysts excreted by *C. cayetanensis* must sporulate in the environment, and become infective after a period of days to weeks. A case study presents examples of intestinal coccidiosis in Polish patients who were diagnosed and treated in the Department and Clinic of Tropical and Parasitic Diseases, Poznan University of Medical Sciences after travelling to tropical and subtropical countries (M. Kłudkowska). The study includes a rare case of co-infection of three species of protozoa in a 32-year-old man who had stayed for about a month in India (*Crypotosporidium* spp., *Giardia intestinalis*, *Entamoeba histolytica*). He reported not following the rules of hygiene and often consumed local food from street stalls. After returning home, signs of gastrointestinal infection such as abdominal pain, nausea, bloody diarrhea (10–15 watery stools per day) and elevated body temperature were experienced. Another study describes an unusual case of isosporidosis in a missionary returning from Chad, Central Africa (A. Lasek – Poznan University of Medical Sciences). The medical history shows that although the missionary spent two years in the village in good living conditions with running water, sanitation and the use of mosquito nets, he ate dishes prepared by his parishioners when visiting them. These presented cases of imported tropical parasitoses suggest that any patient returning from different climatic zones and sanitary-epidemiological conditions should undergo screened for protozoan infections of the digestive system.

Malaria is one of the most severe public health problems worldwide. According to the World Health Organization's World Malaria Report 2014, globally 3.3 billion people (47% of the world's population) live in areas at risk of malaria transmission in 79 countries. It is a leading cause of death in many developing countries, where young children and pregnant women are the groups most affected. In 2014, malaria caused 584,000 deaths (90% of causes were noted in Sub-Saharan African; 78% in children aged under 5 years). Regions associated with the highest estimated relative risk of *Plasmodium* infection for travelers are West Africa and Oceania. Between 2003 and 2011, a total of 189 confirmed malaria cases, including 5 that were fatal, were reported in Poland [50]. All cases were imported and as many as 72% came from Africa. Among cases with a species-specific diagnosis, 118 (73%) were caused by *Plasmodium falciparum*. A recent study presents the epidemiology and diagnosis of malaria in 44 patients hospitalized in 2001–2012 in the Clinic of Tropical and Parasitic Diseases, University of Medical Sciences in Poznan (K. Mrówka, S. Nowak) found the most frequently detected species to be *P. falciparum* (64.8%) and *P. vivax* (20.4%), while mixed infections of *P. falciparum* and *P. vivax* (9%), *P. falciparum* and *P. malariae* (1.9%) were diagnosed much less often. Tourists and work-related travelers (missionaries, contract workers) make up most of the malaria patients in Poland. Their own clinical experiences indicate that detection and measurement of the level of antigen HRP-2 of *P. falciparum* (histidine-rich protein 2 secreted by parasite) in peripheral blood has three important clinical and prognostic values: 1) evaluation of the degree of severity of clinical malaria in patients, 2) determination the risk of multiple organ complications, and 3) monitoring the effectiveness of antiparasitic treatment.

Thanks to the growing population of pet animals, especially homeless dogs and cats, zoonoses still represent a current and serious public health problem. The total number of dogs in the world, regardless of their household status, is estimated at over 500 million. Additionally, the fact that dogs can be a source of more than 60 etiological factors of zoonoses show the scale of the threat to human health posed by animals living in close proximity. In dogs and cats, the most common intestinal parasites are protozoa from the genera *Giardia, Cystoisospora, Sarcocystis, Cryptosporidium*, and the nematodes from *Toxocara, Toxascaris,* *Ancylostoma, Uncinaria, Capillaria and Trichuris* [51]. In addition, cats are the definitive hosts of cosmopolitan protozoan *Toxoplasma gondii*, and dogs of the genus *Echinococcus* tapeworms and nematodes *Dirofilaria* spp. All of the above parasites are zoonotic agents, with *T. gondii*, *T. canis*, *T. cati*, *E. granulosus* and *E. multilocularis* having the most epidemiological importance, being etiological factors of toxoplasmosis, toxocarosis, echinococcosis and alveococcosis, respectively. The development cycle of these parasites is closely related to the environment through their dispersion forms excreted in the faeces of infected animals. Some of these are able to infect humans at the time of excretion (*Cryptosporydium* spp., *G. intestinalis*, *E. granulosus*, *E. multilocularis*), while others become invasive only in the external environment (*T. gondii*, *T. canis*, *T. cati*, *A. caninum*, *U. stenocephala*, *T. vulpis*).

The developmental forms of parasites have been identified in recreational area sandpits in Lodz (J. Błaszkowska, P. Kurnatowski, A. Wójcik, K. Góralska, K. Szwabe – Medical University, Lodz). During autumn 2010 and spring 2013, a total of 68 composite samples of sand taken from 17 sandpits situated in the city of Lodz were tested. Eleven of the sample sites were secured from access to dogs and cats, while the other 6 were not. The average density of *Toxocara* eggs in the unsecured sandpits was 3 times higher than that observed in the sandpits from the secured areas [52]. The number of *Toxocara* eggs recovered decreased following fence construction around the examined children's play areas, but it did not sufficiently prevent contamination. These findings indicate the necessity for educational programmes which should be implemented to protect the local child population from zoonotic infection. Simultaneous initiatives by local communities including the restriction of the stray dog and cat population, hygienic faeces disposal by pet owners and prevention of animal access to play areas may significantly reduce contamination by intestinal parasite eggs.

Until the end of the eighties, the European distribution of *E. multilocularis*, covered five countries (Switzerland, Austria, France, Germany, Russia); however, this number has expanded by a further 11, including Poland [53]. This expansion is associated with increases in the number of foxes, which are the definitive host of this tapeworm. In Poland, the fox population increased 5-fold in the period from 1995 to 2006 and is now estimated at over 200 thousand individuals. In 2009–2013, autopsy examinations revealed the presence of *E. multilocularis* in 16.5% of the intestines of 1546 foxes caught from 16 voivodships [54]. In rural areas, domestic dogs, and occasionally cats, may play a role in the synanthropic cycle of *E. multilocularis*. Humans are accidental intermediate hosts and may be exposed to tapeworm eggs via contact with infected animals, by fruits and vegetables contaminated by the oncosphere or by the environment. Over 1,000 cases of alveococcosis have been reported in Europe, with about 500 incidences in Switzerland. In Poland, 121 cases of alveococcosis were diagnosed in the years 1990–2011 using imaging techniques, histopathology, serological or/and molecular assays [55]. The larvae of *E. multilocularis* most often develop in the liver over many years without symptoms. Due to the lack of a connective tissue capsule intermediate host larval form of the parasite grows in the form, mimicking malignancy. The mortality rate of this parasitosis has been estimated as 70%. The relationship between the presence of eggs in the natural environment and the risk of alveolar echinococcosis were presented (B. Szostakowska, A. Lass, M. Sulima – Medical University of Gdansk). Although transmission of *E. multilocularis* occurs predominantly during the sylvatic cycle, this species may also be spread through rural and peri-urban areas. A recent study found of the presence of *E. multilocularis* DNA in 11.3% of soil samples and 23.3% of samples of fruit, vegetables and mushrooms in rural areas of Varmia-Masuria Province [56,57]. The proportion of positive samples from fruit and vegetables collected from areas close to homes was higher (30.7% – kitchen gardens) than those of samples taken from remote places (20.78% – forests and plantations). These results demonstrate that soil, and the fruit, vegetables and mushrooms intended for human consumption originating in either in the forest and household environment can be a direct source of infection. The successful prevention of alveococcosis demands complex actions comprising public education, control of infection in the wild fox population and the early diagnosis of human population exposed to contact with *E. multilocularis* eggs.

Despite the presence of a zoonotic agent, the *Baylisascaris procyonis* nematode, in the native raccoon population, baylisascaridosis has not yet been noted in Poland. This parasite, like *Toxocara* *canis*, belongs to the order Ascaridida, but its definitive host is the raccoon (*Procyon lotor*), originating from Central and North America. *P. lotor* is an invasive species introduced from the US to Germany and Russia in the 1930s that has since spread to many European countries: France, Belgium, the Netherlands, Denmark, Luxembourg, Switzerland, Hungary, the Czech Republic, Poland, Belarus and the former Yugoslavia [58]. Over 100 species of birds and mammals (especially rodents) can act as paratenic hosts. It is able to develop to maturity in the dog as a nonspecific definitive host. Humans become infected by accidentally ingesting infective *B. procyonis* eggs. An epidemiologic study of *Baylisascaris* infection revealed severe cases of neural larva migrans in infants and young children and ocular larva migrans in adults [59]. Aggressive migration and growth of larvae, particularly within the CNS, can even lead to the death of the intermediate host. Recent attention has been paid to role of raccoons as reservoirs of dangerous pathogens for human and animals, with cases of rabies (Ukraine, Estonia, Germany, Lithuania) and distemper (Germany) being recorded in caught raccoons. Epizootic and zoonotic risks have been demonstrated to be associated with raccoon parasites in Poland (M. Popiołek, A. Piróg, A. Bajon – University of Wroclaw). Coprological analyses of raccoon fecal samples collected in the Warta Mouth National Park situated in western Poland near the Polish-German border revealed the presence of 7 helminth taxa: *Ancylostoma* spp., *Baylisascaris procyonis*, Capillariidae, *Placoconus lotoris*, *Spirocerca lupi*, *Strongyloides procyonis*, and *Echinostoma* sp. [58]. In another study, *B. procyonis* eggs were detected in samples of raccoon faeces collected from areas located in the Słubice district, Lubuskie Province (Poland) [60]. Recently, a parasitological study of soil samples from Kostrzyn upon the Odra River showed the presence of eggs in 8 of 166 (5.2%) examined positions, with between 1 and 8 eggs found per sample (M. Popiołek, A. Piróg, A. Bajon – University of Wroclaw). The prevalence of *B. procyonis* in the examined Polish populations of raccoon is estimated at 1.9–3.7% [58,60,61]. These reports confirm the presence of this dangerous parasite in the Polish raccoon population and the potential risk of infection for local communities.

Serologic prevalence data indicates that toxoplasmosis is one of the most common human infections throughout the world. Most often, horizontal transmission of *T. gondii* to humans is caused either by the ingestion of tissue cysts in infected meat, or food and water contaminated with sporulated oocysts derived from the environment. This parasite may be transmitted vertically by tachyzoites passed to the fetus via the placenta [62]. One clinical form is ocular toxoplasmosis, which can occur in course of congenital or postnatal infections [63]. Ocular lesions may appear often a few months or years after the acute infection [64]. The retina is the primary site of *T. gondii* infection in the eye, but the choroid, vitreous and anterior chamber are also involved. Ocular toxoplasmosis is a progressive and recurring necrotizing retinitis, with vision-threatening complications such as retinal detachment, choroidal neovascularization, and glaucoma [63]. The most common manifestations are focal retinitis, but there can be also present strabismus and nystagmus. A multiannual clinical observation of ocular toxoplasmosis in children hospitalized in the Clinic of Paediatric Infectious Diseases in the Bieganski Memorial Hospital, Lodz, and in The Ophthalmic Ward of the Independent Provincial Hospital, Piotrkow Trybunalski, emphasizes that complete cure is not possible and recommends that patients diagnosed with ocular toxoplasomsis need to be ophthalmically examined at least once every 3 months (K. Dziwińska, M. Raszewska-Steglińska, A. Kuc, E. Majda-Stanisławska). Each patient with inactive retinochoroidal scar requires regular ophthalmic examinations for a lifetime. Although the risk of recurrence was estimated as 50% within 3 years in untreated patients, this figure fell to 15% for patients treated with pyrimethamine. The use of combination therapy (antiprotozoal drug with steroids) was found to result in reduced sizes of retinal scars. Effective therapy of toxoplasmosis requires early diagnosis. Therefore, the identification of new markers confirming the presence of parasites in the host has a high priority. A recent study showed that generated preparations of recombinant ROP5 and ROP18 antigens, expressed in *Escherichia coli* bacteria, are recognized by specific antibodies produced during acute and chronic infections in inbred laboratory mice [65]. The author suggests that ROP5 IgM antibodies are particularly early and sensitive markers of *T. gondii* infection. The high immunoreactivity of the new recombinant ROP antigens gives hope for their use in the diagnosis of human toxoplasmosis and the immunoprevention of *T. gondii* infection as a component of the vaccine.

The obligate intracellular protozoan parasites – *Toxoplasma gondii* and *Neospora caninum* can infect a wide range of animal species. While *T. gondii* is considered an important zoonotic agent and can cause congenital toxoplasmosis and life-threatening infections in immunocompromised persons [66], *N. caninum* is not pathogenic to humans. Although antibodies against *N. caninum* have been detected in humans [67], no reports exist concerning any clinical implications. In veterinary medicine, *T. gondii* is an important cause of abortion in goats and sheep, while *Neospora* infection can lead to fetal loss, especially in cattle [68]. Thus economically, toxoplasmosis and neosporosis are considered important diseases in animals. Recently, the coexistence of *T. gondii* and *N. caninum* in wildlife animals has been evaluated using ELISA assay and PCR (A. Kornacka – Institute of Parasitology PAS, Warsaw). Under the Capercaillie Protection Programme, 119 predator animals such as foxes, raccoon dogs, martens, badgers and American mink have been harvested from the Głęboki Bród forest district located in the Podlasie Voivodeship. Most of the examined animals (97%) had anti-*N. caninum* antibodies, while anti-*T. gondii* antibodies were found in 55%. Additionally, two kinds of these antibodies were detected simultaneously in 52.9% of animals. The results of this study confirm that wild animals constitute an important reservoir of these parasites in the sylvatic cycle in Poland.

Trichinellosis is an important zoonotic infection caused by eating raw or inadequately cooked meat from infected animals. Pigs, wild boar and horses have been identified as main sources of human trichinellosis, which the clinical course may be from asymptomatic to severe. Ten outbreaks of trichinellosis have been reported in Poland in recent decades, mainly in Wielkopolska. During 2003–2014, 244 patients diagnosed with *Trichinella* infection were hospitalized in the Clinic of Parasitic and Tropical Diseases, Poznan University of Medical Sciences (S. Nowak – Medical University, Poznan). The average time of occurrence of symptoms after eating infected meat ranged from 8 to 24 days. It is worth noting that positive serological results (ELISA, Western Blot) were obtained until after 45 days following infection. The intensity of the invasion is the primary factor responsible for the time of onset of symptoms and their clinical course.

In Poland, two *Trichinella* species – *T. spiralis* and *T. britovi*, have been confirmed both in farm (pig, horse) and wild animals (fox, raccoon dog, wolf, marten, badger, nutria, wild boar). The first case of *T. pseudospiralis* invasion was recently reported in red fox hunted in 2012 around Nowy Targ (Malopolska Province) [69], and a second study notes the presence of the arctic species *T. nativa* in one fox from Poland [70]. These observations indicate the possibility of new species of *Trichinella* expanding from neighboring countries to Polish territory. It should be emphasized that the classification of *Trichinella* to species level can be made only by molecular techniques (e.g., multiplex-PCR test). Molecular identification and muscle distribution of *Trichinella* spp. larvae in wildlife carnivores in Poland have been presented (A. Cybulska – Institute of Parasitology PAS, Warsaw). Of the animals harvested from the Głęboki Bród forest district, including raccoon dogs, badgers and martens, the raccoon dogs demonstrated the highest percentage of infections with *Trichinella* spp. (35%), and the dominant species confirmed by multiplex PCR was *T. britovi*. The largest number of larvae was isolated by digestion from the tongue and muscles of the lower foreleg. The results of these studies indicate that the dominant species in the forest environment is *T. britovi* and that both raccoon dogs and foxes are important reservoirs of this parasite.

Invasive diseases with person-to-person transmission, such as giardiosis, enterobiosis, scabies and pediculosis, still constitute significant public health problems. The prevalence of parasites spreading between humans is associated with density of human communities and insufficient level of hygiene. *Enterobius vermicularis* is a cosmopolitan parasite which affects more than 200 million people worldwide [71]. Although this nematode lives in the human large intestine, sometimes the female forms are found in the renal parenchyma, bladder, vagina, ovary and lungs [72,73]. Examples of serious morbidity, such as appendicitis, vaginitis and pelvic inflammatory disease, have been reported as consequences of *E. vermicularis* existing in an ectopic location. This parasite is more common in children worldwide, particularly in the temperate and tropical regions. Enterobiosis is observed most frequently among school children aged 5 to 10 years.

Child care centers are often the site of cases of pinworm infection. The high frequency of *E.*

vermicularis infection among children is associated with specific habits such as geophagia and onychophagia, which substantially increase the risk of infection. In the school year 2002/2003, studies encompassing 31,504 7-year-old children from 15 provinces of Poland showed that the greatest proportion to be infected with intestinal parasites in Warmia-Masuria (29.6%) and the smallest in Silesia (8.8%) [74]. A later parasitological study conducted in the Warmia-Masuria Province between 2003–2006 on a group of children from preschools (9.5%) and orphanages (36.7%) also confirmed a high level of *E. vermicularis* infections [75]. Recently, *E. vermicularis* infections were identified in 10.1% of preschool- and school-aged children, and pupils of childcare centers in Olsztyn. The eggs were detected in 3.9% of preschoolers and pupils, and 32.8% of children from orphanages (K. Kubiak, E. Dzika, J. Korycińska, M. Lepczyńska – University of Warmia and Mazury, Olsztyn). These results showed that children aged 4–9, especially orphans, should be covered by systematic screening with parasitic tests. The promotion of the awareness of *E. vermicularis* infection and their prevention among parents, guardian and educational staff is required.

Parasitic diseases still pose a serious diagnostic and therapeutic problem, especially in the child population. The susceptibility of children to these infections stems from their immature immune system, failure to comply with hygiene rules, specific behavior such as geophagia and onychophagia, and frequent contact with pets. In addition, a key role is played by the degree of parental knowledge on the mode of transmission of parasites. According to Gniadek et al. [76] in Poland, the level of knowledge possessed by parents of preschool children about the risk of parasitic diseases is only satisfactory. Higher levels of parental education were correlated with higher levels of parasitological knowledge. Additionally, women were more knowledgeable in the field of parasitic diseases than men. The financial status of the family did not influence the level of parental awareness. Other studies conducted in Poland indicate that students have a low level of knowledge concerning the parasitological risks for children during their contact with play areas [77]. The degree knowledge of etiological factors of parasitoses is greater among women than men.

Human communities such as kindergartens, schools, nursing homes and hospitals, create favorable conditions for the spread of pathogens. The 2015 report of the Chief Sanitary Inspectorate reports the presence of increasing microbial drug resistance and multi-drug resistance, particularly among strains isolated in hospitals [78]. As many as 7 epidemiological outbreaks of *Klebsiella pneumoniae* resistant to carbapenems were reported in 2014, with these drugs being regarded until recently as "last chance drugs". In total, 401 nosocomial outbreaks were reported to the State Sanitary Inspectorate in 2014, and 394 in 2013. The most frequently reported were infection with *Clostridium difficile* (31% of all infections) and rotavirus (24%). A particularly strong increase was observed in the number of infections concerning the gastrointestinal tract caused by *Clostridium difficile*; these accounted for 11% of nosocomial infections in 2011. It is worth noting that the most common hospital infection found among medical staff was scabies. Although patient infection with scabies has not been subject to reporting to the State Sanitary Inspection since 2009, all nosocomial outbreaks of scabies are recorded, with hospitals reported 15 scabies outbreaks in 2010 and 11 in 2014 [78,79]. The results of a case study indicate that infection with scabies may have an atypical form manifested as subungual changes and can be accompanied by the presence of mold and human grain mites (P. Krzyściak, D. Salamon, K. Talaga – Jagiellonian University, Cracow).

The genus *Ixodes* comprises 241 species and is the largest group of ticks. Ticks may feed on a number of mammalian species, including humans. *Ixodes* sp. Are widely distributed around the world from Europe, Asia up to North America. All stages feed on vertebrate blood, can cause skin irritation and promote a secondary bacterial infection, as well as being vectors of many pathogenic microorganisms. It has been demonstrated that ticks are involved in the transmission of diseases such as tick-borne viral meningitis and tick-borne brain paralysis, Lyme disease, babesiosis and tick-borne hemorrhagic fever [80]. Incidences of viral tick-borne meningitis are falling, with 344 cases registered in 2009, 211 in 2011 and 197 in 2014 [78]. Unfortunately, the Chief Sanitary Inspectorate Report 2015 also notes that the number of cases of Lyme borreliosis has significantly increased in recent years, with 8,783 new cases in 2012, 12,759 in 2013 and 13,875 in 2014, with the incidence exceeding 36/100,000 residents in the final year. Most cases were recorded in the provinces of

Silesia, Podlasie, Malopolska and Mazowsze. Infections with several species of *Borrelia* have been diagnosed: *B. afzelii* – corresponding mainly to atrophic skin changes, *B. garinii* – causing neuroborreliosis and *B. burgdorferi* (in the strict sense) – the etiological agent of borreliosis arthritis.

The high prevalence of infection and identification of infections in areas where they had not previously occurred are associated with the expansion of the range of common occurrence of *Ixodes ricinus* and the migration of animals that are hosts to ticks [78]. Studies conducted in urban areas indicate that the occurrence of ticks in parks, lawns and squares is rising (N. Król, E. Lonc, D. Kiewra – University of Wroclaw; J. Liberska – Adam Mickiewicz University, Poznan). In Wroclaw, the number of ticks feeding on dogs and cats increased from 366 in 2012 to 729 in 2013 and 726 in 2014. From 40 dogs examined in Poznan, 457 ticks were collected. The spread of vectors in the urban agglomeration favors the occurrence of pathogens. In Poland, many individual ticks have been found to be colonized by the spirochete *Borrelia* sp., but also by other groups of pathogens e. g., protozoa of the genus *Babesia*, rickettsia of the hemorrhagic fever group or rickettsia of genus *Anaplasma* (J. Stańczak, A. Stępka – Medical University of Gdansk). In recent years, much interest has been aroused by the rickettsiae of the genus *Anaplasma*, causing human granulocytic anaplasmosis, characterized by high fever, headache and muscular pain and diarrhea, with 17–54% of cases requiring hospitalization. The first case of disease caused by *Anaplasma phagocytophilum* was diagnosed in 1994 in the United States, since then, cases have been reported worldwide with the number increasing substantially [81]. In 2004, a new species was discovered (*Candidatus Neoehrlichia mikurensis*), and in 2010, the first case of infection in Europe was found. The disease usually has a dramatic course and mostly applies to patients with a heavily weakened immune system [82,83]. The high antigenic similarity between the members of the Anaplasmataceae forced the identification of new diagnostic methods based on the analysis of genetic material (E. Gajda, K. Buczkowska-Gawlik, A. Perec-Matysiak, A. Okulewicz, M. Adamczyk, K. Leśniańska, J. Hildebrand – University of Wroclaw). The main reservoir of rickettsial *Anaplasma* are wild rodents, which are hosts of the larval stages of ticks. The spread of ticks to new habitats is encouraged by animal migration and recent gradual changes in climate. Higher winter temperatures compared to those of previous years allow mammals to populate new areas, and the invasive stages of ticks to survive in the new environment [84].

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Received 6 November 2015
Accepted 13 December 2015