Review articles

Parasites and fungi as a threat for prenatal and postnatal human development

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ABSTRACT. Recent literature data reveals the most common etiological agents of congenital parasitoses to be *Toxoplasma gondii*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. An analysis of clinical data indicates that parasitic congenital infections are often asymptomatic, whereas symptomatic newborns usually display nonspecific symptoms, which greatly hinders correct diagnosis. The long-term consequences of prenatal infections are serious clinical problems. This article presents the possible routes of vertical transmissions (mother-to-child) of pathogens including prenatal, perinatal, as well as postnatal routes. It highlights the role of factors involved in protozoa transmission and development of congenital parasitic diseases, such as parasite genotypes, the relationship between the timing of maternal infection and the probability of passage of the parasite through the placental barrier, and the immunological features of pregnant women. Acquired and congenital babesioses in human and experimental animals are presented. It emphasises that the mechanisms by which parasites infect the placenta and cross from mother to fetus are still poorly understood. It also describes the cellular mechanisms of infection by *T. gondii*, such as tachyzoites crossing biological barriers, the expression of Toll-Like Receptors (TLR) family on trophoblasts and syncytiotrophoblasts as an immune response to intrauterine infection and cases of congenital and acquired toxoplasmosis, as well as the long-term consequences of congenital invasion with *T. gondii*, episodes of reactivation of latent toxoplasmosis and *T. gondii* reinvasions. Mycological topics include a rare case of in utero fungal infection of offspring by a mother with vaginal candidosis, and the fungal contamination of ward facilities and medical equipment as potential sources of exogenous infections of newborn children.

Key words: vertical transmissions, congenital parasitoses, congenital toxoplasmosis, placenta, immune responses

Throughout the period of ontogenesis, the human is exposed to infection by various pathogens: viruses, bacteria, fungi and parasites. Some infectious diseases, such as toxoplasmosis, malaria, trypanosomosis and leishmaniosis, are particularly dangerous for pregnant women and their offspring. Parasitic infections affect tens of millions of pregnant women worldwide annually, and directly or indirectly lead to a range of adverse fetal effects, including intrauterine retardation of growth, congenital malformations and even fetal loss. Infections occurring during the first trimester is associated with more severe fetal consequences than those occurring later in pregnancy, which may be asymptomatic. Some of these problems were presented during the 53rd Clinical Day of Medical Parasitology (Lodz, 16th May 2014).

Some infections caused by viruses, bacteria and parasites are more dangerous in pregnant than non-pregnant women because of the potential for vertical transmission. The definition of vertical transmission has a broader meaning in relation to congenital infection as it includes prenatal, perinatal and postnatal routes of passage from mother to offspring, the latter mainly through maternal milk by breast feeding. Congenital infection is a consequence of transmission of live pathogens from an infected pregnant woman to her fetus that persists after birth. Transmission can occur before birth (*in utero* transmission), or at the time of delivery

(perinatal transmission) [1]. Some protozoa (*T. gondii*, *T. cruzi*) can be released into the amniotic fluid (AF) following infection of the amniotic membranes. These parasites might be responsible for complementary oral or pulmonary *in utero* contamination of fetuses bathing in AF and continuously absorbing it. Additionally, congenital infections with *Trichomonas vaginalis* resulting from local contamination of the birth canal at delivery have been described, since this parasite usually colonizes the vagina and the cervix of the female genital tract [2].

Current data on the possible maternal–fetal routes of transmission, the placental responses to protozoa infection and the parasitic factors involved in parasite transmission has been presented (J. Błaszkowska, UM in Lodz). It was underlined that pregnancy changes the functioning of, among others, the reproductive, endocrine and immune systems, influencing the development of parasitic infection and the pathogenicity of the parasites, and the reactivation of invasions in pregnant women. Maternal infections can cause abortion, retardation of intrauterine growth, *in utero* infection of the embryo/fetus and congenital malformations. Parasites capable of congenital transmission are mainly protozoa, such as *Toxoplasma gondii*, *Trypanosoma cruzi*, *Plasmodium* spp., the agents of visceral leishmaniasis, and occasionally, African trypanosomes and *T. vaginalis*, while the transmission of helminths, such as *Mansonella perstans*, *Wuchereria bancrofti*, *Strongyloides stercoralis*, *Ancylostoma duodenale* and *Ascaris lumbricoides*, through the placenta rarely occurs in humans [1].

The mechanisms by which parasites infect the placenta and are passed to the fetus are still poorly understood. The transmission of pathogens through the human placenta from mother to fetus can occur at two sites of direct contact between maternal cells and trophoblasts: firstly, the maternal immune and endothelial cells juxtaposed to extravillous trophoblasts in the uterine implantation site, and secondly, maternal blood surrounding the syncytiotrophoblast [3]. In early pregnancy, the placental barrier reaches a thickness of 50–100 μm and progressively decreases to 2.5–5 μm at the end of pregnancy, facilitating parasite trophoblast crossing. This transplacental route is the most common mode of transmission of parasites with a parasitemic phase (*Trypanosoma* spp., *Plasmodium*

spp., *Leishmania* spp.), as these are present in the maternal blood which bathes the placental intervillous space [1]. The placental barrier must be crossed by the parasites, while *T. cruzi* trypomastigotes remain in the intervillous space and interact with the syncytiotrophoblas. The syncytiotrophoblast forms a surface of about $12m^2$ which remains in contact with maternal blood. Hence, in case of women with Chagas disease, the parasite has the opportunity to interact with a large cellular surface. Assuming that a mean volume of 475 mL/minute of blood reaches the placenta, and that a parasitemia as low as 0.1 to 1 parasite/mL is present, a total of 68544 to 685440 parasites circulate through the placenta in 24 hours [4]. However, parasitemias of over 40 parasites/mL have been reported in pregnant women with acute Chagas disease, implying a total of 27 million parasites may circulate through the placenta in 24 hours. The interaction between human placental calreticulin (HuCRT) showing high expression on placental free chorionic villi and parasitic *T. cruzi* calreticulin (TcCRT) has been recently described. During infection, TcCRT translocates from the endoplasmic reticulum to the area of the emergence of flagella and acts as a virulence factor by binding the maternal classical complement component C1q, which recognizes HuCRT in the placenta, resulting in increased parasite infectivity [5]. These findings suggest that maternal–fetal transmission of *T. cruzi* occurs mainly by the haematogenous transplacental route. On the contrary, it has been observed that *T. gondii* preferentially colonizes extravillous trophoblasts than syncytiotrophoblasts. The overexpression of ICAM-1 on trophoblast and other factors favouring the adhesion of monocytes infected with *T. gondii* can enhance the transmission of parasites to the fetus [6]. This phenomenon can also be observed in case of massive infection with *T. cruzi* associated villitis [7]. However, in the case of infection with *Plasmodium* spp., postulated mechanisms for congenital transmission of malaria parasites include maternal transfusion into the fetal circulation either at the time of delivery or during pregnancy, direct penetration through the chorionic villi, or penetration through premature separation of placenta.

Maternal infection is often associated with pronounced placental inflammation, with an infiltration of neutrophils and lymphocytes

(*placentitis*) that can induce apoptosis in placental cells, and finally rupture of the trophoblastic barrier [1]. This phenomenon has been noted in infections with *T. gondii*, *Plasmodium* spp. and *T. cruzi*. Placentas from mothers with acute Chagas disease, demonstrating a high level of parasitaemia, show severe histopathological changes, such as extensive necrosis, inflammatory infiltration, and amastigote nests [3]. In *ex vivo* conditions, the coincubation of 10^5 or 10^6 trypomastigotes produces infection of the chorionic villi [8]. Additionally, it has been confirmed experimentally that *T. cruzi* induces selective disorganization of the basal lamina, collagen I destruction, and apoptosis, especially in the trophoblast, in infected chorionic villi explants. Moreover, specific changes in the placenta of pregnant women with malaria have been observed. Histologically, placental malaria is characterized by the presence of infected erythrocytes and leucocytes within the intervillous spaces, hemozoin within macrophages, fibrin deposits in trophoblasts and thickening of the trophoblastic basement membrane [9]. A feature of placental malaria is sequestration of *P. falciparum*-infected erythrocytes in the intervillous space. Immunological studies have shown that parasitized erythrocytes adhere to chondroitin sulfate A (CSA) expressed by the syncytiotrophoblast of the placenta. PfEMP1 (VAR2CSA) adhesion antigens exported by *Plasmodium falciparum* parasites to the erythrocyte membrane mediate adhesion to CSA in the intervillous space [10].

Prenatal infections belonging to the TORCH groups of infections represent an important clinical problem for obstetricians, neonatologists and pediatricians [11]. TORCH is an acronym for Toxoplasmosis, Other infections (e.g. malaria, Chagasa disease), Rubella, Cytomegalic inclusion disease and Simplex herpes type 2. These congenital infections share many clinical manifestations, but all can lead to developmental anomalies or even fetal loss. Congenital toxoplasmosis has traditionally been regarded as the most serious outcome of parasitic infection and has an incidence of between 1–15 per 10,000 live births [1]**.** Primary infection in a pregnant woman with the *T. gondii* parasite can have serious consequences on the fetus, ranging from fetal loss to severe neurologic or ocular lesions. In other cases, infected newborns are asymptomatic at birth, but are at risk of developing retinal diseases during childhood or adolescence

from reactivation of latent toxoplasmosis. Important variations have also been observed according to gestational age at the time of maternal infection, with transmission rates about 6% when infection is acquired during the first trimester of pregnancy, and increasing to approximately 22–40% in the second trimester, 58–72% in the third and 90% in the last few weeks before birth [12].

The classic triad of signs suggestive of congenital toxoplasmosis comprising chorioretinitis, hydrocephalus and intracranial calcifications is nowadays rarely observed (10–15% all cases). Clinical manifestations of *T. gondii* infection may be nonspecific [12]. Congenital toxoplasmosis can mimic disease caused by pathogens such as herpes simplex virus, cytomegalovirus and rubella virus. Moreover, the long term consequences of congenital toxoplasmosis may occur after few to several years after birth and most often are associated with damage to the sight and hearing organs. Cases of congenital toxoplasmosis diagnosed and treated in the years 2013–2014 in the Clinic of Pediatric Infectious Diseases in Lodz, have been presented. Moreover, it was described cases of postnatally acquired toxoplasmosis: thirteen cases of ocular toxoplasmosis where the symptoms occurred for the first time, and five episodes where primary infections were reactivated in children aged 2–17 years (A. Kuc; UM in Lodz).

Diagnosis of *T. gondii* infection in pregnant women is particularly important due to the risk of congenital toxoplasmosis. Systematic serological screening for *T. gondii* IgG and IgM antibodies should be performed in all pregnant women in early gestation, i.e. during the first trimester, and in seronegative women each month or trimester thereafter, to allow detection of seroconversion and early initiation of treatment. However, IgM antibodies are not an accurate marker to distinguish between acute and latent infection. Detection of residual or persistent IgM may occur months or even years after primary infection, while the IgG avidity test is a rapid means of identifying latent infections in pregnant women [12]. An analysis of serological tests for *T. gondii* conducted by the Chair of Microbiology of JU-MC in Cracow (D. Salomon and M. Bulanda; Department Epidemiology of Infections, Cracow) revealed *Toxoplasma* antibodies to be present in 295 of 439 women of childbearing age (67%): half of whom had only IgG present, while 15% displayed both G and M class

antibodies. The results of this study indicate that seroprevalence was higher in women residing permanently in rural areas, which may imply a relationship with their living conditions. Poor hygiene, lower socioeconomic status, meat-eating habits, consumption of unwashed raw fruits and vegetables contaminated with the oocytes, as well as easy contact with soil, may also contribute to a higher rate of infection. It should be noted that *T. gondii*, is one of the most common parasites, infecting most genera of warm-blooded animals: more than 30 species of birds and 300 species of mammals [13]. Studies performed by the Institute of Parasitology, Polish Academy of Sciences (A. Kornacka, A. Cybulska, J. Bień, B. Moskwa; Warsaw) in the Głęboki Bród district confirmed that wild animals to constitute an important reservoir of toxoplasmosis in the sylvatic cycle in Poland. High titers of anti-*Toxoplasma* antibodies were found in 95 of 129 examined animals. A high percentage of seropositive foxes was noted. In 16 samples, the presence of *T. gondii* was confirmed by three different tests (ELISA, latex agglutination and PCR assays). A molecular method with TGR1E-1/TGR1E primers revealed the presence of parasite DNA in the brains of 24 animals.

It has been experimentally demonstrated that three genetically-distinct *Toxoplasma gondii* lineages (I, II, and III) have the ability to infect placental explants [14]. Type II is more frequently found in Europe and North America, while genotypes I and III, and recombinant genotypes I/II and I/III have been found in South American and African cases. European and North African epidemiological studies have identified either type II or type I or type I recombinants as being overrepresented in congenital infection, while in Brazil, type I-like strains are more commonly transmitted across the placenta [13].

It is generally assumed that primary infection by *T. gondii* protects from reinfection. However, transplacental transmission may develop after maternal reinfection with different clonal genotypes of *T. gondii* or reactivation of the preexisting disease [15]. Gláucia et al. [16] reported a rare case of congenital toxoplasmosis from an immuno-competent mother with chronic infection who experienced reactivation of ocular disease during pregnancy. Silveira and co-workers [17] describe a case of congenital toxoplasmosis in a newborn whose mother had a 20-year history of a

chorioretinal macular scar and positive serology for toxoplasmosis. Other authors [18] report a case of disseminated congenital toxoplasmosis in a newborn born to a mother who had contact with *T. gondii* strain II before conception. During pregnancy, the mother was reinfected by a highly virulent *Toxoplasma* strain, which is very uncommon in Europe but had previously been described in South America. This incident confirms that acquired immunity against European *Toxoplasma* strains may not protect against reinfection by atypical strains acquired during travel outside Europe or by eating imported meat.

Cellular mechanisms of infection with *T. gondii* have been presented (H. Długońska, University of Lodz). Tachyzoites of *T.gondii* actively penetrate the host cells using parasite motility and the sequential secretion of proteins from such secretory organelles as the micronemes, rhoptries, and dense granules. Experimental studies in mice indicate that both dendritic cells and monocytes/macrophages function as systemic parasite transporters ("Trojan horses") during infection. Examination of peripheral blood taken from patients who have been acutely or chronically infected with *T. gondii* has demonstrated tachyzoites circulating both as free forms or within peripheral blood mononuclear cells [14]. The parasite can be transmitted from infected dendritic cells to NK cells. Dissemination of the parasite from the primary infection site, typically the gut epithelium, occurs via intracellular and extracellular mechanisms. *T. gondii* can flow freely in host fluids, migrate along cell layers (endothelium, epithelium), cross them (paracellular route) or use motile host cells as vehicles (intracellular route) to reach distant organs [19]. It has been recently reported that tachyzoites of *T. gondii* are able to cross structural-functional biological barriers, such as the blood-brain, blood-eye barriers and the placenta, leading to the respective development of neurotoxo-plasmosis, ocular toxoplasmosis and congenital toxoplasmosis of the host [14,20]. The paracellular transmigration of *T. gondii* tachyzoites is based on the sliding motion (gliding motility), the response of the MIC2 parasite proteins and of the ICAM-1 host adhesion molecule. It was found that extracellular tachyzoites can also pass polarized cell monolayers (paracellular transmigration). These forms of the *T. gondii* genotype I, which is highly virulent to mice, enter the bloodstream as free cells or paracellularly, whereas those of genotype II, those

with low virulence towards mice, mainly move by intracellular transmigration.

Recent studies have shown that 10 members of the Toll-Like Receptors (TLR) family are expressed by trophoblast and syncytiotrophoblast. TLR-2 and TLR-4 recognize pathogen-associated membrane patterns of *T. gondii*, *T. cruzi*, and *P. falciparum* [1]. Such TLR expression is strongly enhanced during placental infection [21]. In addition, the contribution of placental barrier, *T. gondii* genotypes and SNPs of TRL genes in the course of primary infection in pregnant women have also been presented (W. Wujcicka ICZMP, Lodz). During pregnancy, the changing frequency of transplacental transmission of *T. gondii* from 0% in the case of infection before pregnancy to about 67% for infection between 31 and 34 weeks of pregnancy may reflect the gestational age-dependent expression level of TLR. The restricted expression of TLR in trophoblast cells during the first trimester of pregnancy may indicate a lower ability of early placental cells to engage the immune response to infection and intrauterine infection. Differential expression of TLRs depends on placental cell types and gestational age [22]. The expression of TLR2 and TLR4 in the first trimester of pregnancy has been reported only for villous cytotrophoblasts and extravillous trophoblasts, but not for syncytiotrophoblasts (ST), which suggests that placental tissue and the fetus might be infected by pathogens which have passed through the breached TLR-negative ST. The involvement of single-nucleotide polymorphisms (SNPs) in the TLR genes in infectious pathogenicity, including *Toxoplasma* retinochoroiditis, points at the possible involvement of TLR. Wujcicka and coworkers [22] emphasise the necessity for further studies of TLRs in the maternal–fetal transmission of particular parasite strains and congenital toxoplasmosis.

The antibiotics sulfadiazine and pyrimethamine remain the most effective treatments for toxoplasmosis despite their known side effects, including bone marrow suppression and liver toxicity. However, efforts are being made to identify new agents to inhibit proliferation of *T. gondii* in infected patients. One area of research is based on the range of plant compounds which exhibit anti-protozoal efficacies, including an anti-*T. gondii* effect. A number of herbs have been used for many years as human anti-parasitics in Asian countries. *In vitro* experiments indicated that *Sophora flavescens*

and *Zingiber officinale* extracts, which show highly selective toxicity against *T. gondii*, may represent sources of new anti-protozoal drugs isolated from plants [23]. These medicinal herbs appear to be more efficacious against this parasite than pyrimethamine and sulfadiazine in *in vitro* cell culture. In the other hand, increased proliferation of *T. gondii* strain RH was recently observed in the presence of phytoecdysteroids from *Arcangelisia flava* (20-hydroxyecdysone, ecdysone) in human peripheral blood monocytes, regardless of the serological status of the donor (K. Dzitko, J. Gatkowska, B. Dziadek, M. Antczak, H. Długońska, University of Lodz). Hence, phytoecdysteroids should not be considered as potential drugs against *T. gondii*. In addition, these compounds commonly occurring in foods in respectively highly doses may increase proliferation of tachyzoites and the development of parasitic infection.

Although helminth larvae and schistosome ova have been found in the human placenta, congenital transmission of helminths is not common [1]. A few cases of transplacental passage of the microfilariae of *Onchocerca volvulus*, *Wuchereria bancrofti* and *Mansonella persten* have been reported [24]. Congenital parasitic infections such as ancylostomosis, trichinellosis and ascariosis have been recorded more rarely [25]. It has to be noted that transmissions of helminths through placenta are limited to only species which have a developmental cycle based around the migration of larvae in host blood. Numerous experiments on laboratory animals have confirmed the possibility of vertical transmission of *Trichinella* larvae both through the placenta to the fetus and with the milk of the mother to offspring [26].

Cases of congenital trichinellosis in humans have been reported sporadically. The infection of a woman with *T. britovi* at the 10th week of gestation was reported to lead to transplacental transmission of larvae to the fetus. At the request of the mother, an abortion was performed in the 22nd week of pregnancy. In the placenta, fetal tissues and organs, larvae were found in amount between 0.02 and 30 per gram of tissue [27].

The rare reporting of congenital parasitic diseases may presumably result from the low incidence of infection in humans. The European Food Safety Authority (EFSA) European Centre for Disease Prevention and Control (ECDC) reports that nematodes of the genus *Trichinella* caused 301

confirmed human infections in the European Union in 2012. The EU notification rate was 0.06 cases per 100,000 population and trichinellosis was most frequently reported in Latvia, Lithuania, Romania and Bulgaria: these four countries accounting for 82.4 % of all confirmed cases reported in 2012 [28]. Of the four species of *Trichinella* described in Europe (*T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*), three: *T. spiralis*, *T. britovi* and *T. pseudospiralis* are found in wild animals in Poland [29]. As the parasite is more prevalent in wildlife, especially wild boar, than farmed animals, the unexamined meat of hunted animals is the main source of trichinellosis in the country. As a result of a study based on 793 wild boar samples from various regions of Poland taken in the period 2011–2014, the Institute of Parasitology, Polish Academy of Sciences (A. Cybulska, A. Kornacka, J. Bień, W. Cabaj, B. Moskwa, Warsaw) reported 17 animals (1.8%) to be infected with *Trichinella* spp. (*T. spiralis* 65%, *T. britovi* 33% and *Trichinella* spp. 2%).

The increasing number of cases of infection with *Babesia* sp. in the past decade has resulted in increased interest in this parasite (E. Gołąb, NIPH National Institute of Hygiene, Warsaw). The first case of human infection was detected in 1957 in Yugoslavia, and since then, four species have been found to be pathogenic to humans: *B. microti*, *B. divergens*, *B. duncani* and *B. venatorum*. The vectors of infection are ticks, mainly *Ixodes scapularis* and *Ixodes ricinus* [30]. The disease may be asymptomatic or demonstrate nonspecific flu-like symptoms with a very high body temperature. Peripheral blood parasitemia is 10–20%. Approximately 84% of infections are found in patients after removal of the spleen, with the parasitemia in these cases be as high as 80% [31]. However, congenital babesiosis caused by maternal infection with *Babesia microti* transmitted by *Ixodes scapularis* as a vector has been rarely reported [32]. Until now, many similarities were noted in cases of congenital babesiosis, including asymptomatic maternal infection and development of fever, hemolytic anemia, and thrombocytopenia in infants detected between 19 and 41 days after birth. All infants required a blood transfusion because of severe anemia. The developmental forms of *B. microti* are presumably acquired antenatally, similarly to *Plasmodium* spp., by transplacental transmission of infected erythrocytes. The clinical

symptoms for prenatal infection with *Babesia* are similar to those of congenital malaria in non-disease endemic areas. The laboratory animal studies performed by an intercollegiate team from the University of Warsaw and Warsaw University of Life Sciences (K. Tołkarz, A. Bajer, A. Rodo, R. Welc-Falęciak and M. Bednarska; UW, Warsaw University of Life Sciences) indicated the presence of reproductive disorders and pathological changes as a result of fatal infection with *B. microti*.

Approximately 9–10% of nosocomial infections are fungal infections, with 85.6% of these caused by fungi of the genus *Candida*, and mortality due to invasive candiosis is 40–70%. Infection is most common in Intensive Therapy and Oncology units, with the respective rates being 16.1 and 15.6 per 1000 hospitalized individuals. On the other wards fungal infections are less common: 5.8 per 1000 for surgery, and from 0.2 to 1.3 per 1000 for Gynaecology and Obstetrics [33]. On average, 29% of nosocomial infections are transmitted through the hands of medical staff. Other cases are related to the contamination of air, usable space and equipment in health care facilities [34,35]. In neonatal units, fungi are detected on 60% of equipment surfaces: the walls of the incubators, respiratory masks, breathing tubes and mattresses from incubators. Mould fungi such as *Penicillium*, *Cladosporium* and *Aspergillus* predominate, while species of *Candida* represent only 10% of isolated fungi (A. Gniadek, A. Bialec-ka, I. Opach, A. Kulig, A.B. Macura – Jagiellonian University, Cracow).

Infants are at risk of infection with fungi during the perinatal period, especially those born prematurely with low birth weight. As many as 60% of newborns with a birth weight below 1500 g are colonized by fungi during the first month of life, while 20% of these develop fungal sepsis. The occurrence of fungemia increases the risk of death in premature children from 7% to 28% [36]. Recurrent vulvovaginal candidosis applies to 40-50% of women of childbearing age and 25% of pregnant women, and neonatal infections usually occur during the perinatal period [37]. Infection occurs vertically only in rare cases, when the placenta, umbilical cord, and in special cases, the fetus, are occupied (M. Skoczylas, PUM, Szczecin). The occurrence of amniotic invasion can lead to premature delivery, and neonatal sepsis [38]. In prospective studies, including premature neonates with very low birth weight, it has been shown that

the use of prophylactic doses of nystatin or fluconazole significantly reduces the risk of death due to invasive candidosis [39,40,41]. Some authors recommend the use of antimycotics in neonates belonging to groups at high risk for candidemia: those receiving antibiotic therapy in the first 4–6 weeks of life, those with low birth weight or those whose mothers suffer from chronic vulvovaginal candidosis [36,40,41]. Unfortunately, no information exists concerning the effect of such prophylaxis on the state of the natural microbiota of the child.

Systemic fungal infections with fungemia are an important diagnostic problem for doctors and medical analysts. Rapid detection of pathogens facilitates therapy, shortens the duration of treatment and often reduces the risk of death. Classical culturing methods unfortunately are long-term: yeast culture requires a minimum of 24–48 hours, while mould and dermatophyte culture need as long as 1–4 weeks [42]. However, the time for diagnostics can be significantly reduced by the use of molecular or serological methods. Existing methods are based on detection of fungal cell wall components in blood, urine or cerebrospinal fluid. In cases of invasive aspergillosis – galactomannan, a polysaccharide characteristic of fungi of the genus *Aspergillus*, has proven to be valuable. Mannan, the main component of the cell wall of *Candida,* is used as a marker for invasive candidosis, and 1,4-β-glucan for cryptococcosis. These polysaccharides can be detected by latex agglutination assays or immunoenzymatic reactions. Unfortunately, due to possible cross-reactions on antigens of bacteria or other types of fungi, false positive results are possible [43], while a low concentration of the antigen in the blood can lead to false negative results. Similar diagnostic difficulties may appear in parasitic invasions (J. Matowicka-Karna; UM Białystok). Serological methods are used mainly in infections by helminths such as *Ascaris* sp., *Toxocara* sp., *Echinococcus granulosus*, *Taenia saginata* and *Schistosoma* sp., as well as by infections by such protozoa as *Trypanosoma* sp., *Toxoplasma gondii* or *Entamoeba histolytica*. An indirect immunofluorescent antibody assay can be used to diagnose *Babesia microti* with 88–96% sensitivity and 90–100% specificity. Microscopic examination is also important in the diagnosis of malaria, babesiosis, leishmaniosis, Chagas disease, sleeping sickness and microfilarosis. Thin blood smears or thick drop preparation can be used to determine the level of parasitemia in cases of malaria and babesiosis. The detection of developmental forms of *Plasmodium* in the blood ranges from 41–93% depending on the method of staining: quantitative buffy coat method, acridine orange or benzothocarboxypurine staining [44,45]. However, detection of parasites in microscopic preparations requires extensive experience and a high level of perception on the part of the diagnostician.

In parasitic and fungal invasions, interactions between the pathogen and host cells play a major role. Depending on the efficiency of defense mechanisms, the pathogen may be removed, incorporated within the ontocenosis as a commensal, or may act as the causative agent of the disease [46]. The immune response of the host organism can be stimulated by various elements of the structure and function of the parasite or fungus. One of the important factors may be the different types of proteins secreted by pathogens, which may be proteins secreted by micronemes, rhoptries and dense granules in cases of protozoal infections. While proteins secreted by the micronemes are responsible for the initial attachment, proteins secreted by dense granules and rhopries are responsible for subsequently modulating the host cell to form a habitat for the parasite [47]. The rhoptry proteins play a particularly important role in the case of *Toxoplasma gondii* infection. Rhoptry proteins ROP18 and ROP5 are involved in the formation of parasitophorus vacuoles in which tachyzoites are able to develop [48]. The team from the University of Lodz (M. Grzybowski, B. Dziadek, H. Długońska) showed that immunization with recombinant proteins ROP18 and ROP5 stimulated humoral and cellular response in laboratory animals.

Chitin, a polymer of N-acetyl-D-glucosamine, plays an important role in the pathogenesis of multicellular parasites and fungi (Doligalska, Brodaczewska, Donskow-Łysoniewska – University of Warsaw). It is the main component of the cuticle of helminths and fungal cell walls. Its formation is regulated by chitin synthetase, which occurs in the fungal cell wall and so plays a major role in the growth and differentiation of the hyphae [49, 50]. The formation of the hyphae by fungi inhabiting the mucous membrane promotes the penetration into host tissues and increasing the enzymatic potential of the cells. The free chitin

particles in the environment can stimulate a cellular immune response [51].

Pregnancy can be disrupted at the maternal, fetal and placental levels by parasitic invasion. Congenital infections with *T. gondii*, *L. donovani* and *T. cruzi* parasites can also lead to serious chronic infections later in adult life. Often asymptomatic at birth and remaining undetected (mainly Chagas disease, visceral leishmaniosis), these congenital infections are more common than expected. Despite major advances in prenatal and postnatal diagnosis, congenital parasitoses still represent a serious health problem for offspring. Maternal infections can lead to fetal malformation or fetal loss. It should be emphasized that the estimated number of infants born every year with prenatal parasitic infection is significantly undervalued. The absence of any objective assessment of the incidence of congenital parasitoses is the result of diagnostic problems in live-born infants, sporadic autopsy of aborted fetuses and late manifestation of clinical symptoms of congenital parasitic disease. Understanding the mechanisms by which parasites infect the placenta and pass into the fetus is one of the most important topics that will help find specific biomarkers of infection, prevent congenital transmission, maintain the health of the newborn, and develop efficient treatments.

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