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Acanthamoeba – pathogen and vector of highly pathogenic bacteria strains to healthy and immunocompromised individuals

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Abstract

Acanthamoeba is a free-living protist pathogen, which is present in every place on Earth. 50 to 100 percent of the adult population has serum antibodies, specific for Acanthamoeba antigens. Acanthamoeba is an etiological agent of keratitis and encephalitis diagnosed in human. Acanthamoeba keratitis occurs in healthy persons and may lead to visual impairment and blindness, because corneal infection with this parasite fails to induce cell-mediated immune response due to the absence of resident antigen-presenting cells in the cornea. Systemic immunization with Acanthamoeba antigens induces Th1 cell-mediated immunity and serum IgG antibody, but do not prevent the development of keratitis. Immunization via mucosal surfaces stimulates IgA antibodies in tears and protects against the development of keratitis.

Amoebae feed mainly on bacteria, fungi, and algae. By transferring intracellular bacteria, amoeba contributes to the spread of diseases dangerous to humans. Some microorganisms have evolved to become resistant to protist, since they are not internalized or able to survive, grow, and exit free-living protists after internalization. In many cases, the bacteria inside living amoebae survive longer, and multiply better, showing higher virulence. There is a hypothesis, which assumes that Acanthamoeba and symbiontic bacteria survive and multiply better in moist soil, rich in nitrogen compounds, particularly in the vicinity of the root systems of Alnus glutinosa, infected with nitrogen-fixing bacteria Frankia alni. Impact of soil environment created by nitrogen-fixing bacterium Frankia alni on specific relations between protists Acanthamoeba and highly pathogenic bacteria strains in Alnus glutinosa habitats in Poland continue to be established.

Key words: *Acanthamoeba, endocytobionts, Coxiella burnetii, Francisella tularensis, immune response, Alnus glutinosa.*

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Introduction

Protists from *Acanthamoeba genus* are a group of free-living organisms within a cosmopolitan range. In their life cycle are trophozoites and cysts. Until now, these organisms were isolated from various natural environments, artificial environments, and from tissues and body fluids of animals and humans [1].

These parasites are etiological agents of many human diseases such as encephalitis and primary meningitis, granulomatous inflammation of the brain, inflammation of the cornea, and amoeba-induced inflammation of many organs [2-4]. Over the last years, an increase in number of immunocompromised people and alarmingly high resistance of invasive forms of protozoa to routinely used disinfectants were observed as well as growing number of diseases related to their presence in water. The virulence markers include both activity of specific proteolytic enzymes and increased presence on the surface of the cell membrane of mannose-binding proteins (MBP), allow adhesion [5-7]. Granulomatous amebic encephalitis and disseminated infections occur in persons with a compromised immune

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system. Acanthamoeba keratitis occurs in healthy persons and may lead to visual impairment and blindness, because corneal infection with this parasite fails to induce cell-mediated immune response due to the absence of resident antigen-presenting cells in the cornea [8]. Infection with amoebas is recognized by Toll-like receptors and induces both innate and adaptive immune responses. Systemic immunization with *Acanthamoeba* antigens induces Th1 cell-mediated immunity and serum IgG antibody, but do not prevent the development of keratitis. Immunization via mucosal surfaces stimulates IgA antibodies in tears and protects against the development of keratitis, mainly through inhibition of parasites binding to corneal epithelial cells without affecting their viability. Also, IL-17A production after *Acanthamoeba* infection plays an important role in host protection, through increased migration and activation of neutrophils. Bacterial flora of ocular surface exacerbates the course of Acanthamoeba keratitis by forming endosymbionts with parasites [4, 9-11].

Protists from *Acanthamoeba* genus act as vectors of pathogenic microorganisms (bacteria, viruses, fungi, and *Protista*), contributing to the spread of diseases dangerous to humans and animals. In many cases, the protists - bacteria symbionts are more resistant to damaging agents, and inhabiting bacteria survive longer, better, and multiplying higher virulence [12, 13]. This affects many highly pathogenic microorganisms, including bacteria from genus *Mycobacterium*, *Shigella*, *Salmonella*, and *Yersinia*, and species from *Bacillus anthracis*, *Vibrio cholerae*, *Legionella pneumophila*, *Francisella tularensis*, and *Coxiella burnetiid* [14-20]. Greub and Raoult [12] verified amoebae as a reservoir of these bacteria, naming as "Trojan horse" responsible for the spread into the environment.

In general, forest soil samples contain 104-107 of active protist individuals per gram of dry soil and litter. Abundances of other soil organisms vary in depth through the profile, with gradients of organic matter and physical properties. These values fluctuate daily with changes in moisture, temperature, and food abundance [16].

Soil protists contribute to organic matter decomposition and mineralization, or to the detritus food-web, through several trophic functional groups. The structure and function of the soil food-web were recently reviewed [17]. Many soil protists are bacteriovorous. Bacteriovores consist of species that ingest bacteria by phagocytosis. In selecting bacteria as prey, some bacteriovores are less discriminating than others. In some cases, ingested prey bacteria contain toxins, which cause lysis of consumers, such amoebae. There are many species of amoeba-resistant bacteria, including *Francisella tularensis* (cause of tularemia) and *Coxiella burnetii* (cause of Q fever) [1, 15, 18-20].

In recent years, roles of *Acanthamoeba* as reservoirs, hosts, and vectors for endocytobionts were investigated. The term "endocytobionts" refers to bacteria, fungi, small protozoa or viruses, which are able to reside permanently or transiently in the cellular milieu of the amoebae [21]. Cellulose-rich wall of *Acanthamoeba* protects their endocytobionts from external toxins, involving factors produced by immune cells.

Acanthamoeba play an important role in the functioning of natural ecosystems, because of its impact on the structure of bacteria communities and circulation of organic substances in natural environment [1]. The abundance and amoebae species diversity in the soil environment are influenced mainly by the time of year, temperature, humidity, rainfall, soil pH, and availability of nutrients [22]. There is a high probability that the amoebae are willing to elect soil rich in nitrogen, such as communities of alder (*Alnus glutinosa*), which coexists with nitrogen-fixing bacteria from the *Frankia alni* species. This bacterium is in a symbiotic relationship with the *Alnus* and forms nodules on the tree's roots [23]. Possible connection between the micro-environment of root system of black alder and the outbreaks of tularemia in rodents can be observed based on the results of the work conducted in Volyn by Professor Adamovich [24-26].

The protists from *Acanthamoeba* genus were tested in Poland as an etiologic agent of human disease, or as a component of natural and artificial ecosystems [4, 27-29]. We did not find a study concerning the influence of environmental factors on the nature of interaction (symbiosis, parasitism) with *Acanthamoeba* and bacteria, and high virulence in humans and animals, such as *F. tularensis* (cause of tularemia), and *C. burnetii* (Q fever source). These diseases, some of the most dangerous infectious diseases in humans and animals, have been discovered in the early twentieth century. Most of tularemia cases among European countries have been reported in the Scandinavian region [30]. There is a danger of *F. tularensis* use as a biological weapon agent [31]. The main sources of tularemia infection are hares, rabbits, small forest and field rodents, wild birds, and water. The main vectors can be food, sucking blood insects, ticks, and amoeba. Natural tularemia infection in the world was found in 190 species of mammals, 23 species of birds, 3 species of amphibians, and 3 species of fish [32]. In the past, tularemia epidemics developed also through hamsters purchased in pet stores and through domestic animals (mainly cats).

Cell-mediated immunity is recognized as a critical for protection against *F. tularensis*. Furthermore, researchers' findings suggest that humoral immunity plays a significant role in the elimination of *F. tularensis* by interaction with cell-mediated mechanisms. This immune interaction provides prophylactic and post-exposure protection against this pathogen [33, 34].

In Poland, the first information about tularemia was described in 1931 by Prof. J. Brill, but the first case of tu-

laremia was diagnosed in 1949 [30]. Prof. E. Skrodzki was the first to deeply investigate tularemia and who described the epidemiological situation of tularemia in Poland until 1971, which shows quite frequent developing of tularemia in various Polish regions. Microbiological analysis of *F. tularensis* was also performed by Prof. Świętosław Bilecki. Current research on the presence of *F. tularensis* in Poland focused on the analysis of serological assays based on people coming from regions suspected of tularemia occurrence in their area.

Etiological agent of tularemia is a gram-negative bacteria *F. tularensis*. Due to the high genetic similarity within the species *F. tularensis*, 4 subspecies have been specified, including *F. tularensis* subsp. *tularensis* (type A or so-called nearctica), *F. tularensis* subsp. *holarctica* (type B, formerly known as *palearctica*), *F. tularensis* subsp. *mediasiatica*, and *F. tularensis* subsp. *novicida*. Each subspecies has been isolated from different regions of the world, and type A is the most frequently isolated in the USA (although in 1998, its presence was found in Slovakia), type B is the most frequently isolated in Europe, subsp. *mediasiatica* in Asia, and subsp. *novicida* in Australia. The genus *Francisella* also includes species *F. philomiragia* (non-pathogenic to humans, usually isolated from fish) and *F. noatunensis* [35-37]. It is worth mentioning that in the last years, a study detected the occurrence of *F. tularensis* in ticks, mites, fleas, and small mammals in Slovakia, Czech Republic, and Germany. More recently, the method has been successfully used in MST for genotyping strains of *F. tularensis* in a local epidemic of tularemia in France, resulting in the description of several new genotypes previously identified [38-41].

Q fever is a disease of domestic and wild animals that can also infect humans. The disease is caused by the *C. burnetii* bacterium, which is very resistant to chemical and physical factors.

Coxiella burnetii was described for the first time in the thirties of the twentieth century in Australia. The presence of this factor was found practically all over the world as evidenced by local epidemics (the last took place in 2007 in the Netherlands).

In Poland, the highest incidence of Q fever among livestock and people were described in 1982 at the South-Eastern Polish border. Then antibodies against *C. burnetii* in 220 cows and 1,300 people were found. Until now, this was the largest Q fever epidemic in the world [42].

First incidence of Q fever was described in 1956 in the village Owczary (Nowy Sącz province) [43]. Later, Q fever occurred throughout the territory, but it should be noted that due to the very uncharacteristic symptoms, it could not be diagnosed properly.

In Europe, the epidemiological situation and the epizootic incidence of *F. tularensis* and *C. burnetii* are relatively well understood. The use of molecular biology methods allowed for in-depth characterization of the agents and to determine their types and MST genetic profiles, which has also been linked to their geographical occurrence [44-46].

Etiological agent of Q fever is a gram negative rod, measuring from 0.2 to 1 μm, occurring in two morphologically different forms, such as small cell variant (SCV) – spores, which are highly resistant to physical and chemical factors, and large cell variant (LCV) – a form of metabolically active and sensitive to environmental change. In addition, Q fever can take two forms of the disease (acute as highly contagious, dependent on the presence of phase II *C. burnetii*) and chronic form (phase I antibodies).

Two forms of *C. burnetii* can be detected by serological methods, such as IFA and ELISA. The first form detected is the virulent phase, known as phase I with smooth LPS. The second form is the phase II, which have rough LPS. Defined titers of phase I and phase II antibodies allow to distinguish acute from chronic form of Q fever and is a predictor indicating the evolution from acute to chronic form. For many years, the serological diagnosis of humans was based on two highly specific tests, such as complement fixation test (CSF) and microagglutination test, in phases I and II simultaneously performed. Other methods, including Western-blotting and radioimmunological assays, have also been applied in diagnostics of Q fever [46-50].

Both cellular and humoral immunity are important in the host defense against intracellular bacteria of *C. burnetii*. The humoral immune response (measuring the level of IgM and IgG specific antibodies in phase I and II) may be useful for an early detection of *C. burnetii* in infected animals. In relation to cell-mediated immune response, it is the crucial immune component for protection of a necessary intracellular pathogen by persistence within phagocytic cells and not distinguished between *Coxiella*-infected and non-infected pregnant animals [51, 52].

Conclusions

Till now, protists from *Acanthamoeba* genus were studied in Poland as the etiologic agent of human diseases or as a component of natural and artificial ecosystems [4-7, 27-29, 53-57]. Moreover, in our country, no study was published describing the impact of environmental factors on the occurrence of amoebae in the soil of different forest communities as well as the impact of these factors on interactions between protists and endosymbiotic bacteria. Soil environment contains a great amount of information. In the future, studies should be carried out based on a detailed micro-polygons investigation of the natural environment. Micro-polygons could be determined by the root system of *Alnus glutinosa* infected with nitrogen-fixing bacterium *Frankia alni*, and in control areas characterized by nitrogen compounds deficiency in soil. A coincidence of outbreaks of tularemia and alder forests was found in Ukraine [24-26].

The impact of soil environment created by nitrogen-fixing bacterium *Frankia alni* on specific relations between protists *Acanthamoeba* and highly pathogenic bacteria strains in *Alnus glutinosa* habitats in Poland, continues to be established.

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