TRYPANOSOMIASIS IN MICE OF THE CHERNOBYL ZONE OF RADIOACTIVE CONTAMINATION

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Abstract. One of the most obvious and long-lasting consequences of the Chornobyl nuclear power plant accident is the creation of an exclusion zone and a zone of unconditional (compulsory) alienation. The effect of succession is manifested in the fact that a significant part of aquatic and terrestrial ecosystems as of 1986 was artificial or semi-artificial systems that were under regulatory control by humans. Parasitic systems are one of the informative indicators of processes occurring in ecosystems. Any changes in the host population lead to changes in the parasite population. The degree of imbalance of the "parasite-host" system

depends on the nature and strength of the influence of external factors. In order to study the parasitic systems of the exclusion zone as informative indicators, rodents were selected. Among the rodents studied for the presence of causative agents of parasitic diseases were field (Apodemus agrarius) and yellow-necked (Apodemus flavicollis) mice. A study of them was conducted for the presence of causative agents of blood-borne parasitic diseases.

Examination of mice blood smears revealed the presence of causative agents of blood parasitic and infectious diseases in all of the investigated animals. Among the causative agents of these diseases, Trypanosoma spp., Babesia spp., Rickettsia spp. and others were identified. Trypanosoma spp. was identified for the first time on the territory of Ukraine in blood smears of 3 mice among investigated, which were 25 % (95 % confidence intervals 6.8–54.1). The number of parasites in 200 fields of view of the microscope from the studied mice varied from 2 to 25.

Keywords: hemoparasitic diseases, mice, Apodemus flavicollis, Apodemus agrarius, Trypanosoma spp.

Introduction

One of the most obvious and long-lasting consequences of the Chornobyl nuclear power plant accident is the creation of an exclusion zone and a zone of unconditional (compulsory) alienation. Here, the negative impact of radioactive contamination on humans and the environment has manifested itself to the maximum. This led to the evacuation of the population and the curtailment of economic activity in an area of 2.600 km². 90-95% of this area has no systematic human activity and the regime is equal to the reserve (Balashov et al., 1992). Ecosystems formed in the exclusion zone are influenced by several key factors. Among them - radioactive contamination, succession, and climate change.

A characteristic feature of radioactive contamination of Chornobyl origin is the spatial heterogeneity of density, radionuclide composition, and physicochemical forms of precipitation. Most of the radionuclides were part of the "hot particles". The largest "hot particles" fell in the near zone. Contamination of most of the zone territory was formed by the finely dispersed fraction of "hot particles". In the form of radioactive contamination outside the ex-

clusion zone, the condensation component is dominated. Today, the radiation situation is formed by the following elements: ¹³⁷Cs, ⁹⁰Sr, Pu (isotopes 238, 239, 240), ²⁴¹Am.

The effect of succession is manifested in the fact that a significant part of aquatic and terrestrial ecosystems as of 1986 was artificial or semi-artificial systems that were under regulatory control by humans. These are agro-landscapes, monocultural forests, irrigation and drainage systems, inhabited localities. The removal of regulatory control has led to the inclusion of natural mechanisms of dynamics, including catastrophic (fires, outbreaks, floods, etc.) (Vishnevskiy et al., 2004). That is, a significant part of ecosystems is in a state far from equilibrium, where the processes of succession are active.

The testamentary effect is the result of a radical reduction in economic activity and the creation of a strict protection regime. The increase in the number of background species and the appearance of rare species of animals and plants has been recorded for several years after the accident. We should also take into account the high landscape diversity and a large area of the exclusion zone.

Climatic factors are external to natural complexes and do not depend on them. In recent years, climate change has had a significant impact on the hydrological regime and vegetation.

Based on the above, we can say that the dynamics of ecosystems in the exclusion zone is unique. The need for its study is due to a number of reasons: management of water and forest resources, assessment of the significance of natural complexes, the study of parasitic systems and zoonoses, and so on.

Parasitic systems are one of the informative indicators of processes occurring in ecosystems. Any changes in the host population lead to changes in the parasite population. The degree of imbalance of the "parasite-host" system depends on the nature and strength of the influence of external factors (Semenko et al., 2017; Semenko et al., 2020).

In order to study the parasitic systems of the exclusion zone as informative indicators, rodents were selected. Among the rodents studied for the presence of causative agents of parasitic diseases were field (*Apodemus agrarius*) and yellow-necked (*Apodemus flavicollis*) mice.

Analysis of recent research and publications

Trypanosomoses are diseases, which are caused by parasites of the genus *Trypanosoma*. They could be extracellular (*Trypanosoma evansi*) and intracellular (*Trypanosoma evansi*) parasites (Riedel, 1975; McGhee et al., 1980; Nagle et al., 1980; Rodriguez-Morales et al., 2011; Norman et al., 2013; O'Connell et al., 2015). Species of this genus may differ in their transmission types (Sato et al., 2003; Rocha et al., 2004; Morio et al., 2008; Rotureau et al., 2013). Species, which are independent of tsetse flies belong to non tsetse transmitted animal trypanosomoses (NTTAT) (Schaub et al., 1994; Sharma et al., 2009). Investigation of these para-

sites, their control measures are coordinated with OIE Non Tsetse Transmitted Animal Trypanosomoses Network according to support of a global strategy for the control of NTTAT. NTTAT are spread in Africa, Asia, Latin America, and Europe.

We haven't found information about the spreading of these parasites on the territory of Ukraine. But on the territory of Russia, causative agents of breeding disease of one-hoofed animals (*Trypanosoma equiperdum*) and surra of horses and camels (*T. evansi* (Fig. 1)) have been registered (Georgiu, 2015). *T. evansi* may cause in experimentally infected mice splenomegaly and hepatomegaly (Ghaffar et al., 2016) and might be a reason for human disease (Joshi et al., 2005).

Trypanosome species that are transmitted without tsetse flies include *T. evansi*, *T. equiperdum*, and *T. vivax*. These species cause surra, dourine, and nagana in a wide range of domestic animals like cattle, buffalo, horse, camel, small ruminants, and others. All these diseases lead to economic losses in endemic areas (Hoare et al., 1972; Stevens et al., 1999; Powar et al., 2006; Otto et al., 2010; Schwan et al., 2016). And also, these species could parasitize in the body of rodents such as mice.

One of the species, which could parasitize in the body of rodents and is transmitted by fleas is Trypanosoma (Herpetosoma) lewisi. Animals are infected by the oral route, through contamination by flea feces or ingestion of fleas (Shaw et al., 1973; Guerrero et al., 1997; Desquesnes et al., 2002). Trypanosoma musculi is also transmitted between mice by ingestion of infected fleas. These trypanosomes are of similar morphology, making it difficult to distinguish them by microscopy. There are also few records of human infection cases caused by T. lewisi (Viens et al., 1972; Ponzi et al., 1984; Howie et al., 2006; Rayat et al., 2014; Hong et al., 2017).

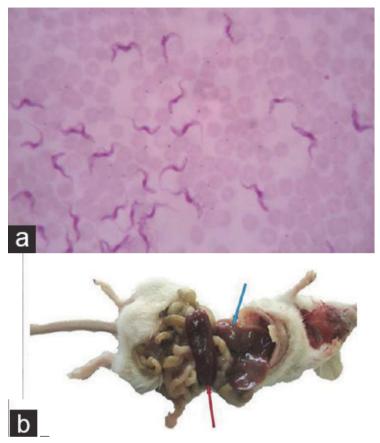


Fig. 1. (a) Giemsa stained blood smears from mice infected with T. evansi, ×1000; (b) Splenomegaly (red arrow) and hepatomegaly (blue arrow) in experimentally infected mice with T. evansi (Ghaffar et al., 2016)

3 morphologically indistinguishable subspecies of *Trypanosoma brucei* are involved in a complex transmission cycle between humans, tsetse, and reservoir hosts. These subspecies cause Gambian and Rhodesian sleeping sickness or coexist with the other trypanosomes in reservoir hosts and vectors (Dumas et al., 1999). And according to the results of investigations, *T. cruzi* DNA has been identified in mummified human beings in South America dating back 4000 years, which means that human beings were exposed to African trypanosomes concomitantly with their evolution (Guhl et al., 2000; Barrett et al., 2003; Otero et al., 2012).

Methods for identification of trypanosomes include rodent subinoculation, cryopreservation, *in vitro* methods, isoenzyme electrophoresis, molecular characterization, population genetics methods, true phylogenetic methods, and others (Dumas et al., 1999).

Pentamidine, suramin, melarsoprol (late-stage disease with central nervous system disorders), eflornithine are licensed for the treatment of trypanosomiases in humans. Nifurtimox, although only licensed for use against Chagas' disease. Diminazene (berenil), a veterinary medicament, has also been used occasionally without a license (Pepin

et al., 1994; Barrett et al., 2003; Pereira et al., 2009; Shikanai-Yasuda et al., 2012). But such species as *T. vivax*, according to the research, is resistant to diminazene aceturate but sensitive to isometamidium chloride (Desquesnes et al., 1995).

To the principles of control of trypanosomiases belong treatment of infected individuals, active case finding with mobile teams, vector control, personal prophylaxis, and serological control of blood banks (Barrett et al., 2003).

Materials and methods of research

The research was carried out on the 2^{nd} landfill (51 ° 22'20.60 "N 30 ° 8'26.94" E) located on the drained areas at the bottom of the cooling reservoir of the Chornobyl nuclear power plant. The strength of the exposure dose of γ -radiation and the flux density of β -particles above the soil surface in places of catching animals were determined by γ -, β -radiometer "Pripyat" RKS 20.3.

Catching murine rodents of various species, which are widely used in radiobiological studies as indicators, were carried out in the second half of September 2020 using traps of the Sherman system. The duration of catches at the landfill was 3 days with daily inspection of traps. After capture, the animals were taken to the laboratory of the Institute for Nuclear Research of the National Academy of Sciences of Ukraine (Kyiv, Ukraine), examined, registered with individual numbers and values of the date and place of capture, species name, gender, body weight. Species affiliation of individuals was determined by morphological characteristics. Age was determined by a set of features: weight, body length, development of reproductive organs, and thymus. Thin blood smears were prepared from a drop of blood obtained from the heart. Staining of smears and their further examination was carried out on the basis of the Department of Pharmacology, Parasitology and Tropical Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. A set of Leucodif 200 (LDF 200) kit in accordance with the manufacturer's instructions was used in order to stain the smears. Examination of stained blood smears was performed using a microscope with ×1000 and ×1150 magnification.

Results of the research and their discussion

12 mice were caught using traps of the Sherman system and then investigated. Among them, 6 mice were field (*Apodemus agrarius*) and 4 – yellow-necked (*Apodemus flavicollis*). For two mice, the species was not identified.

6 mice were females and 5 – males. For one mouse, age was not identified.

According to the blood smear examinations from mice of Chornobyl Radioecological Biosphere Reserve, *Trypanosoma spp.* agents were revealed for the first time in Ukraine (Fig. 2, 3). They were identified in blood smears from 3 mice, which accounted for 25% (95% confidence intervals 6.8–54.1). 2 of 3 positive mice were males *Apodemus agrarius* and 1 was unidentified gender *Apodemus flavicollis*.

The number of parasites in 200 fields of view of the microscope from the studied mice varied from 2 to 25.

Sizes of the $\emph{Trypanosoma spp.}$ in blood smears were 30–40 μm in length.

Also during blood smear microscopic examinations, *Babesia spp.* was identified in 8 mice (66.7%; 95% confidence intervals 37.7–88.4), *Rickettsia spp.* – in 9 mice (75%; 95% confidence intervals 45.9–93.2); anisocytosis, polychromasia, poikilocytosis, basophilic granularity, plasma cells, reactive lymphocytes, Jolly little bodies, and hypochromic erythrocytes were observed.

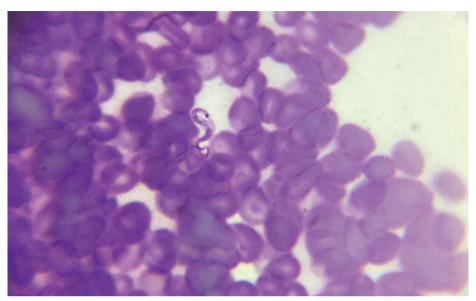


Fig. 2. Leucodif 200 (LDF 200) stained blood smears from mice infected with $Trypanosoma\ spp., \times 1000$

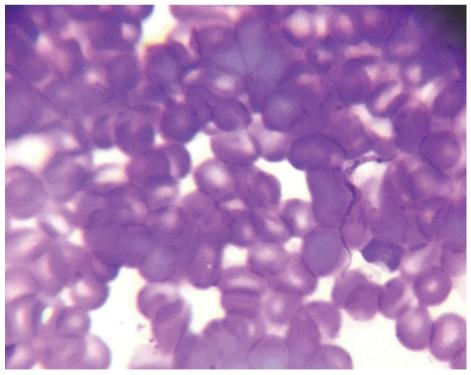


Fig. 3. Leucodif 200 (LDF 200) stained blood smears from mice infected with $Trypanosoma\ spp., \times 1000$

Conclusions and future perspectives

This is the first identification of the *Try-panosoma spp*. in Ukraine. Thus, murine rodents are hosts for a significant number of blood parasites, both infectious and parasitic. Some of them are also causative agents of zoonotic diseases. Further research, including PCR investigation, is essential to identify species of these agents, possible routes of transmission to murine rodents, and risks of transmission from animals to humans. In our future investigations we plan to conduct also genetic analysis to establish the registered species of *Trypanosoma spp.*, *Babesia spp.*, and *Rickettsia spp.* not only morphologically in blood smears.

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Анотація. Одним із найбільш очевидних і довготривалих наслідків аварії на Чорнобильській атомній електростанції стало створення зони відчуження та зони безумовного відчуження. Ефект сукцесії проявляється в тому, що значна частина водних і наземних екосистем станом на 1986 рік були штучними або напівштучними системами, які перебували під регуляторним контролем людини. Паразитоценози можуть бути інформативним фактором процесів, що відбуваються в екосистемах. Будь які зміни в популяції хазяїв призводять до змін у популяціях паразитичних організмів. Рівень співвідношення у паразито-хазяїнних системах залежить від сили і впливу зовнішніх факторів. З метою вивчення паразитарних систем зони відчуження як інформативних показників було відібрано гризунів. Серед досліджуваних мишей на наявність збудників паразитарних захворювань були польові (Ародетиз аgrarius) та жовтогорлі (Ародетиз flavicollis). Було проведено їхнє дослідження на наявність збудників кровопаразитарних захворювань.

Дослідження мазків крові мишей виявило наявність збудників паразитарних та інфекційних захворювань крові у всіх досліджуваних тварин. Серед збудників цих хвороб були виявлені Trypanosoma spp., Babesia spp., Rickettsia spp. та інші. Trypanosoma spp. було вперше виявлено на території України в мазках крові 3 мишей серед досліджуваних, які становили 25% (95% довірчі інтервали 6,8–54,1). Кількість паразитів у 200 полях зору мікроскопа досліджуваних мишей коливалася від 2 до 25. Трипаносомози тварин і людини є надзвичайно поширеними. Збудники-трипаносоми спричиняють багато різних хвороб великої рогатої худоби, коней, верблюдів, дрібної рогатої худоби, зокрема, сурру, дурину, нагану та інші. Крім того є причиною гамбійської й родезійської сонної хвороби людини.

Ключові слова: кровопаразитарні хвороби, миші, Apodemus flavicollis, Apodemus agrarius, Trypanosoma spp