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TROPHIC CHAIN YERSINIA PSEUDOTUBERCULOSIS

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Abstract: *This paper tries to reproduce two types of trophic chains on the example of an aquatic ecosystem model, with the participation of planktonic and benthic organisms: "yersinia - infusoria - daphnia - fish" and "yersinia - insect larvae - fish".*

The concentration of Yersinia pseudotuberculosis during primary infection (infusoria culture medium) was 10^9/ml. In the first hours after infection, the concentration of bacteria in the infusoria decreased (10^5cfu/mass). In daphnia, which consumed infected infusoria, the concentration of Yersinia pseudotuberculosis decreases in the first 3-5 days (from 10^5 to 10^3 cfu/10 individuals), then the reproduction of Yersinia pseudotuberculosis in daphnia continues, and on the 11th day it reaches 10^7 cfu/10 individuals.

The concentration of Yersinia pseudotuberculosis in the intestines of fish fed infected daphnia (10^7cfu/10 individuals) is continuously decreasing: 10^5cfu (after 24 hours) and 10^2-10^1cfu/individuals (after 15-30 days).

Key words: *daphnia, ecosystem, infusoria, yersinia, food chain, fish*

INTRODUCTION

Interactions between genus populations in the ecosystems of natural foci of infection are complex and diverse. Unlike aboveground, terrestrial and aquatic ecosystems are poorly studied in this respect: certain papers are devoted to the analysis of interactions between a large number of pathogenic bacteria and specific hosts, primarily protozoa. Modeling of trophic chains, and even bacteria, in the experiment, although it represents a certain simplification, it allows to challenge and analyze the probability of transmission of the infectious agent in the community, to determine specific routes of its circulation, changes in bacterial population at different levels of the trophic pyramid. the potential epidemiological significance of certain links in these chains.

There are almost no special manuscript in the literature on the migration of pathogenic microorganisms along the trophic chains of aquatic and terrestrial communities; only certain assumptions in this regard are known. Thus, for example, Liston (1990) believes that bacteria and toxins reach fish through trophic chains. Shubin (1993) states that the circulation of pseudotuberculous microbes in natural foci is significantly related to water and hydrobionts: infection of fish and fish-eating birds can be done through food and water throughout the year. Đukić (2011) points out that in the population of yersinia found in the community with infusoria in clay-swamp soil extract, after the first day there is a certain increase in the number (10^6) of yersinia, after which their concentration decreases to 10^5 cfu/cm^3 on the third day and the eighteenth - up to 10^3cfu/cm^3). According to the authors, water and hydrobionts take second place (after the soil and its inhabitants) as reservoirs of pseudotuberculous microbes in natural hotspots.

The hydrobiotic organisms are potential natural hosts of *Y. pseudotuberculosis*, and fish as the final link of some alimentary chains, could present epidemic danger as a food product for human consumption (Pushkareva et al., 1994). Gengler et al. (2015) state that entomopathogenic nematodes are an efficient reservoir that provides exponential multiplication, maintenance and dissemination of *Y. pseudotuberculosis.*

Yersinia strains are psychrotrophic bacteria that are resistant to many environmental factors (Triantafillidis et al., 2020). *Yersinia pseudotuberculosis* is present in the soil, in fresh farmproduced plants and root vegetables, but also has numerous animal reservoirs and is abundant in wildlife, including birds, rodents, rabbits, deer, dogs, cats, cattle and insects and amoeba in the environment (Brady et al., 2022; Martínez-Chavarría et al., 2015)

This paper is an attempt to reproduce two types of trophic chains on the example of an aquatic ecosystem model - with the participation of planktonic and benthic animals: "yersinia - infusoria - daphnia (cyclops) - fish" and "yersinia - insect larvae - fish".

MATHERIAL AND METHODS

The work was performed in the public health institutes of Čačak and Kraljevo in 2007 year. Axenic culture of *Tetrahymena pyriformis* infusoria was infected with pseudotuberculous microbe at a concentration of 10^9 cells/ml. After incubation for 1-2 hours at 22-25 ºC, the infusoria biomass was collected on filters ("Millipore", pore diameter 6 micrometers) and washed from extracellular bacteria; with bacteriological control of the rinsing water. In aquariums with daphnia (cyclops) infusoria with phagocytic yersinia were introduced, in a concentration that is close to natural - 10^3-10^4 cells/1l of water. In this way, the infected lower shells are cultivated. Cyclops were used to infect fish, which "consumed" infected tetrahymen for one day-up to the peak concentration (in them) of pseudotuberculous microbes (10^5 cfu/100 individuals). Daphnia were kept for 7-11 days after feeding with infected infusoria, after which yersinia reached their maximum concentration (10^7 cfu/10 individuals).

Each fish (guppies) was infected individually in cups (once), giving it 10 infected daphnia or 100 cyclops as food (with magnifying glass control). Bacteriological examinations were performed during the first week once a day, and then twice a week.

Table 1. *Yersinia pseudotuberculosis* abundance at different trophic levels during "infusoria-daphnia-fish" chain transmission

Trophic chain links	Time limit (day)	Yersinia concentration	
		"At the entrance"	"At the exit"
Infusoria	1	10^9/ml (in the middle)	10^5 (in infusoria)
Daphnia	1 to 5	10^5cfu/10 individuals	10^3
	7 to 11	10^5	10^7
Fish	1	10^7 cfu/10 individuals	10^5
	15 to 30	10^3	10^2-10^1

RESULTS AND DISSCUSION

In our research, in the process of migration of *Yersinia pseudotuberculosis* along food chains, changes in its number at different trophic levels were found (Table 1). In the infusoria culture medium, the yersinia concentration was 10^9/ml during the primary infection. Infusoria phagocytosed a significant number of bacteria from the environment: after two hours, intense phagocytosis was determined, with each tetrachimene unit containing from several to 30-40 digestive vacuoles filled with yersinia. In the first hours after infection, the concentration of bacteria in the infusoria was 10^5 cfu/biomass and remained unchanged during the day.

In daphnia, which consumed infected infusoria, in the first 3-5 days, a decrease in the concentration of *Yersinia pseudotuberculosis* from 10^5 to 10^3 cfu/10 individuals was observed, after which the reproduction of yersinia in daphnia dominated in relation to their digestion, so an increase in numbers was observed up to 11 days (10^7 cfu/10 individuals). Fish that received daphnia as food with the maximum number of yersinia (10^7 cfu/10 individuals), kept them for 30 days (observation period), but the concentration of the pathogen in the fish intestine was continuously decreasing: initial - 10^7, after 24 hours - 10^5, after 15-30 days - 10^2-10^1 cfu/units (Table 1).

In the links of the trophic chain "yersinia - infusoria - cyclops - fish" a slow decrease in the concentration of *Yersinia pseudotuberculosis* was observed - in the consumption of infected infusoria by cyclops and infected cyclops by fish. However, as with the consumption of daphnia, the fish retained the pathogen in the intestine for 30 days (observation, monitoring period) at the level of 10^2-10^1 cfu/individual.

The diet of fish with chironomid larvae previously infected with pseudotuberculous microbes (10^6 cfu/individuals) also led to infection of fish: the concentration of bacteria in the intestines of aquarium fish stabilized at 10^4 cfu/individuals for 15 days, and then to 30-th day decreased to 10^1 cfu/individuals.

Therefore, the first attempts to experimentally reproduce the migration of yersinia along the trophic chains of the aquatic ecosystem, with the participation of zooplankton (lower crustaceans), benthos (chironomids) and nekton (fish) showed that pseudotuberculous microbe, along with using various transmissions across different food chains of the community from lower trophic levels to higher ones, with natural trophic networks being complex (Figure 1).

It is obvious that yersinia reach the organism of freshwater and transient fish (even those used for human consumption), from where pseudotuberculous microbial cultures have been isolated many times (Shubin, 1993).

According to study of Santos-Montanez et al. (2015) *Y. pseudotuberculosis* is able to resist the bacterivorous nature of free-living amoeba *Acanthamoeba castellanii* and have an enhanced ability to replicate and persist in coculture with amoeba.

In these experiments, we tried to give indicative numerical estimates of the process of yersinia migration in the community. The ability of pathogenic microorganisms to exist in water and soil and to reach humans through the food chain, indicates that research on the circulation of pathogens in the environment is of great importance for human health.

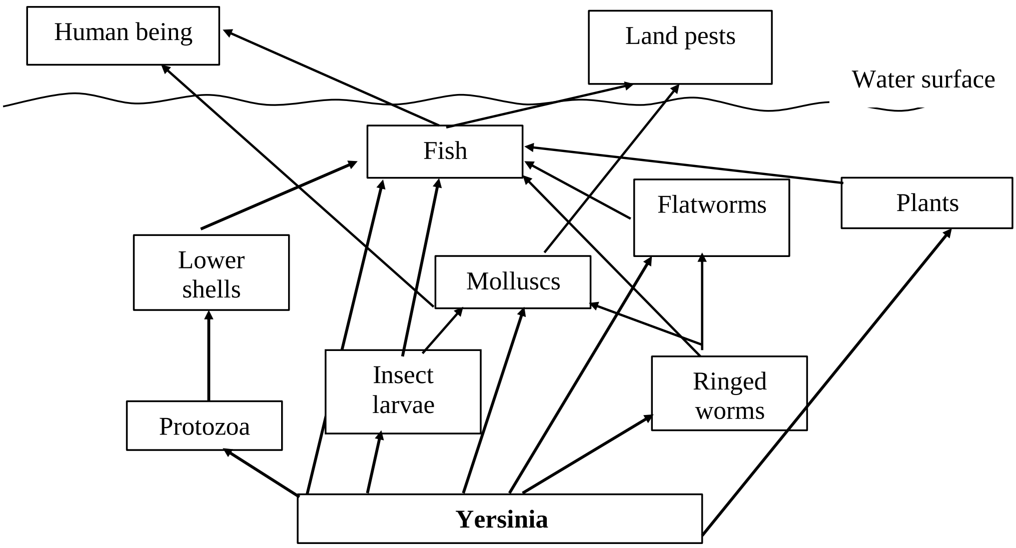

Figure 1. *Y. pseudotuberculosis* migration pathways by trophic chains
experimentally determined pathways
other possible pathways

CONCLUSION

The patterns and mechanisms of circulation of infectious agents in terrestrial and aquatic ecosystems are just beginning to be studied, because the attention of researchers has traditionally been focused on aboveground ecosystems - natural focus zoonotic infections. Now, the specificity of the natural focus of sapronoses is becoming more and more important, above all - the ecology of the challengers as full members of land and water communities. A wide perspective of field and experimental research in this relatively new direction of research on the problem of natural focus of infectious diseases opens up. The ability of pathogenic microorganisms to exist in water and soil and to reach humans through the food chain, indicates that research on the circulation of pathogens in the environment is of great importance for human health.

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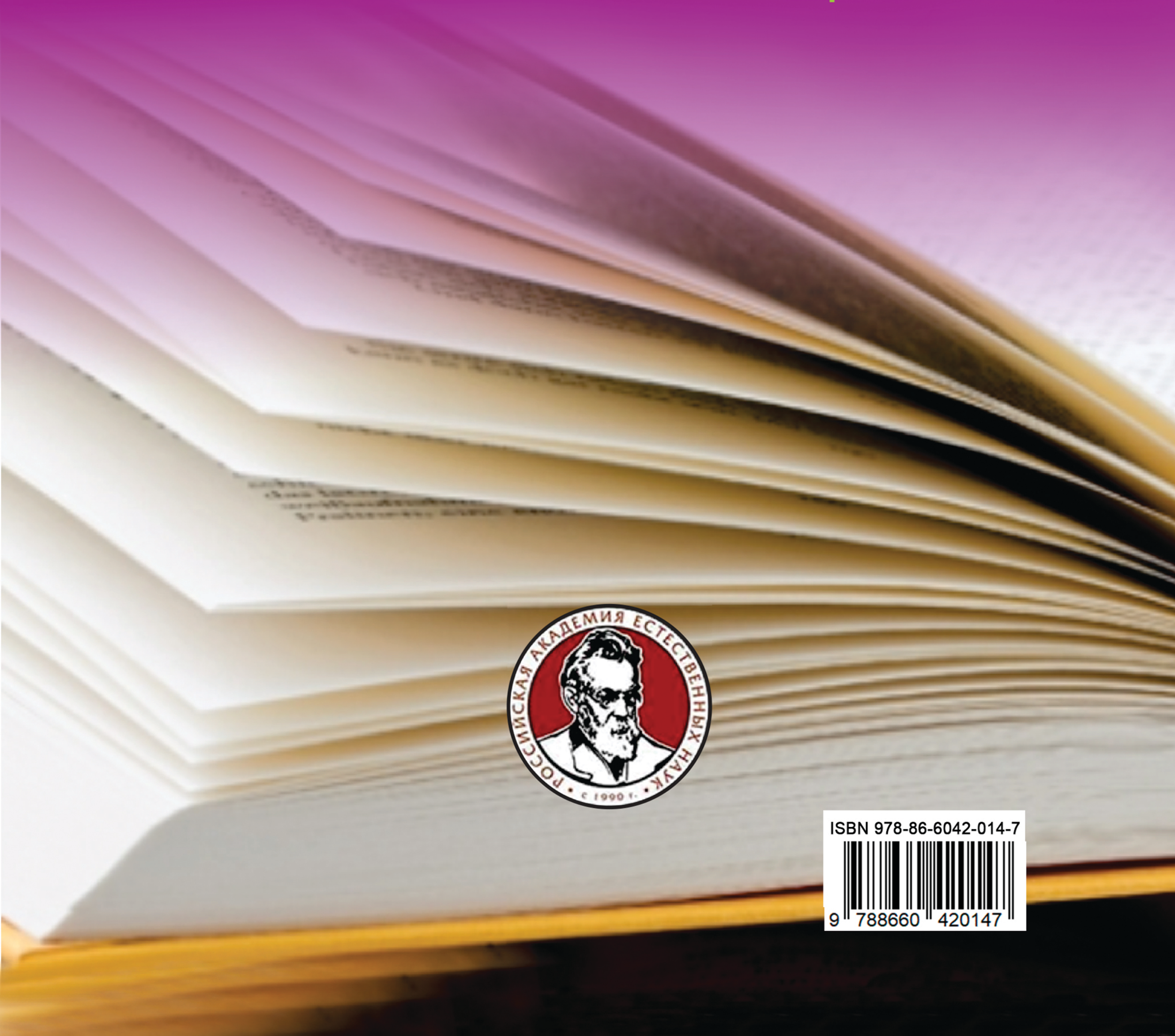

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