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Role of the lowest shellfishes *Daphnia magna* in existence of pathogenic bacterium *Erysipelothrix rhusiopathiae*

Objective. To establish the ability of lower crustaceans Daphnia magna to maintain the existence in the aquatic environment of pathogenic bacteria Erysipelothrix rhusiopathiae - causative agents. Methods. Daphnia was infected by bacteria E. rhusiopathiae through water. During the experiment, the bacteria content in the crustacean and water organism was determined. Results In the experiment, planktonic crustaceans D. magna were infected with E. rhusiopathiae bacteria through water. Pathogenic bacteria isolated from the body of Daphnia for 20 days, and from control samples - 6 days. Conclusions Lower crustaceans D. magna are capable of sustained time - 20 days (observation time) to maintain the presence of pathogenic E. rhusiopathiae bacteria.

Key words: Erysipelothrix rhusiopathiae, Daphnia magna, duration of existence.

Among a variety of various dangerous infectious agents, a special group includes pathogens of sapronoses, in particular, pathogenic bacteria Erythiocynum rhusiopathiae. The ability of E. rhusiopathiae to persist for a long time and actively develop in various objects of the environment (soils, reservoirs) creates considerable difficulties in the prevention and elimination of outbreaks of diseases caused by microorganisms of this species [2, 7 - 11]. Being part of the aquatic and soil ecosystems, E. rhusiopathiae bacteria interact with the various components of these groups - prokaryotes, protists, higher plants, and animals. The consequence of these environmental relationships can be both the preservation and reproduction of the pathogen, and the reduction of its size or even destruction [5]. To study the effects on the survival of bacteria E. rhusiopathiae in the aquatic environment, we selected crustacean species Daphnia magna - a classic object of biological and environmental research. The literature data indicate that D. magna sustained the existence of a pseudotuberculosis agent (Yersinia pseudotuberculosis) for a long time, and also eliminated the spores of the pathogenic fungus Batrachochytrium dendrobatidis [8]. The purpose of the research is to establish the ability of lower crustaceans D. magna to support the existence of E. rhusiopathiae in pathogenic bacteria in the aquatic environment. Materials and methods of research. Laboratory culture D. magna was received at the Institute of Hydrobiology of the National Academy of Sciences of Ukraine. The crustaceans were kept in glass aquariums with a volume of 5 dm3 at a temperature of +22 .. + 24 ° C, as a feed used by the alga culture of chlorella and baker's yeast. Two strains of E. rhusiopathiae of different virulence bacteria, obtained from the Institute of Veterinary Medicine of the National Academy of Sciences (Kyiv), were used in the studies. The highest virulence rate was inherent in the strain 149-LD50 for white mice was 1, 4 ± 0.2-10 KUO per head. The experiments also used avirulent strain of BP-2 var. IVM, which did not lead to the death of mice at a dose of more than 1 to 10 bacteria in the mice. The cultures of E. rhusiopathiae were grown on a cardiovascular broth (AES Chemunex, France) at a temperature of + 36.7 ± 0.3 ° C for 48 hours. The bacterial content was recorded by seeding the samples from the experimental and control samples in dilutions 1-10-1, 1-10-2, 1-10-3, 1-10-4 on the surface of the selective agar-aged Parker medium containing crystalline- Summer and sodium azide [8]. After cultivation at temperature (+ 36,7 ± 0,3) ° C, for 72 hours, the number of colonies that were grown was calculated and the average number of colonies of the forming units (CFU) of bacteria per 1 cm was determined. Experimental samples contained a culture of Daphnia, which was fed strains of bacteria E. rhusiopathiae. Control - similar in volume aquariums without crustaceans with inoculated strains of bacteria.

Research results and their discussion. During the first 2 days, the bacterial content decreased when D. magna was infected with E. rhusiopathiae virulent strain through water. Starting from the 3rd day of the experiment, the bacterial content in daphnia gradually increased and reached the maximum at the 12th day - 3.7 ± 0.4-106 CFU / person, while their concentration in water was quite low - 1.4 ± 0 , 1-10 CFU / cm3. Subsequently, the number of E. rhusiopathiae in daphnia gradually decreased and by the 20th day from the

beginning of the experiment was 7.4 ± 0.2-102 CFU / individual, in water the bacterial content was 8.0 ± 0.2 ^ 101 CFU / cm3. In control samples, the concentration of bacteria from the beginning of the experiment was sharply reduced and after 6 days, E. rhusiopathiae was not microbiologically detected in water (Fig. 1). A comparison of the number of daphnia suggests that, at the initial concentration of crustaceans in specimens of 15 - 20 individuals / dm3, at the end of the experiment, their content in the experiment was 18-26 individuals / dm3, and in the control - 32 - 40 individuals / dm3 (some daphnia specimens were extracted during the experiment To determine the content of bacteria). Detection in experimental specimens of rabbits D. magna cultures of E. rhusiopathiae of the aurulent strain BP-2 var. IVM caused a sharp decrease in the content of bacteria during the first 5 days. In the future, the E. rhusiopathiae concentration in daphnia was fairly low and kept with minor fluctuations at a level of 5.1 ± 0.2 ^ 101 KOU / individual until the 8th day, after which the bacteria were no longer allocated (Fig. 2). From the control samples bacteria were not bacteriologically allocated to the 3rd day from the beginning of the experiment. There are no distinct differences in the density of Daphnia cultures in the samples at the end of the experiment: we tested 29-37 individuals / dm3; Control - 32 -40 individuals / dm3. The results of the studies indicate that E. rhusiopathiae bacteria are able to infect crustaceans D. magna through water and stored in them for 20 days. The increase in the number of bacteria in Daphnia from the 3rd to 12th day of the experiment for their low content in water indicates that E. rhusiopathiae are not simply stored in the body of crustaceans (probably gastrointestinal tract), but also actively multiply. By isolating the bacteria in water, the infected Daphnia maintain their number in the external environment, since in the control samples (without crustaceans) the E. rhusiopathiae content declined quite rapidly. The virulence of the E. rhusiopathiae strain is of great importance for the infection and storage periods of bacteria in D. magna. Thus, the bacteria of high-strain strain 149 secreted from the organism the crustaceans of the entire observation period (20 days), while the bacteria of the aurulent strain BP-2 are var. IVM was detected only during the first 8 days of the experiment.

Fig. 1. Dynamics of the content of bacteria E. rhusiopathiae virulent strain 149 in conjunction with crustacean D. magna: 1 - E. rhusiopathiae in daphnia; 2 - E. rhusiopathiae in water (experiment); 3 - E. rhusiopathiae in water (control) (for figs 1 and 2)

Fig. 2. dynamics of the content of bacteria E. rhusiopathiae avirulent strain BP-2 var. IVM in conjunction with crustacean D. magna

The ecological relationship between crustaceans D. magna and E. rhusiopathiae bacteria is not limited to trophic bundle of the "predator-victim" type. Bacteria with a high level of virulence can stay and multiply in the body of D. magna for a long time, which makes it possible to conclude that there is a formation of a "parasite-nutrient" type of trophic link. Under natural conditions, lower crustaceans can be filtered from the environment E. rhusiopathiae. Bacterial cells that have a low level of virulence are likely to be digested, while virulent ones find a favorable environment for survival and move to residence in the body of D. magna. By interaction with D. magna in the heterogeneous E. rhusiopathiae population, virulent bacteria can be preferred, which ultimately leads to an increase in the proportion of such cells in the total mass and an increase in the virulence rate of the entire population. Consequently, planktonic crustaceans, in particular D. magna, can play an important role in the presence of pathogenic E. rhusiopathiae in conditions of fresh water. The obtained data should be taken into account during the development and implementation of measures for the prevention and control of diseases in the wilderness of farm animals, as well as during the remediation of water from pathogenic bacteria E. rhusiopathiae.

Conclusions

Pathogenic bacteria E. rhusiopathiae are capable of infecting crustacean D. magna through water and storing it for 20 days (observation period). The level of virulence of bacteria E. rhusiopathiae is of great importance for the infection and storage periods of bacteria in D. magna. In the organism infected Daphnia bacteria E. rhusiopathiae multiply and stand out in the environment, resulting in their concentration in water. In interaction with crustacea D. magna in the populations of E. rhusiopathiae, a high level of virulence can be maintained.

Bibliography

1. Biological activity of cuttings of Percidae family fish in relation to bacteria Erysipelothrix rhusiopathiae / O.V.Gulai, O.M. Zhukovsky, V.V. Gulay, N.P. Tkachuk // Fishery science of Ukraine. - 2015. - No. 2. - P. 61 - 68.

2. Borisovich Yu.F. Infectious Animal Diseases: Directory / Yu.F. Borisovich, L.V. Kirillov; Under Ed. DF Osidze - Moscow: Agropromizdat, 1987. - 288 p.

3. Gulay O.V. The role of riverine birds of the genus Podiceps in the ecology of bacteria Erysipelothrix rhusiopathiae / O.V. Gulay, O.M. Zhukorsky // Fishery science of Ukraine. - 2015. - No. 1. - S. 26 - 33.

4. Ostroumova I.N. Biological Basics of Feeding Fish / I.N. Ostromovom - SPb .: Publishing house of the State Social Research and Development Institute "GosNIORH", 2012. - 564 p.

5. Epidemiological aspects of the ecology of bacteria / V.Yu. Litvin, A.L. Ginsburg, VI Pushkareva et al. - M .: Pharmarus Print, 1998. - 255 pp.

6. *Buck J.* Predation by zooplankton on Batrachochytrium Dendrobatidis: biological control of the deadly amphibian chytrid fungus / J. Buck, L. Truong, A. Blaustein // Biodiversity and Conservation. - 2011. - No. 20. - R. 3549-3553.

7. Cussler K 100 years of erysipelas prophylaxis: significance and reduction of animal experiments / K. Cussler, E. Balks // ALTEX. - 2001. - V. 18, No. 1. - P. 29-33.

8. Packer R.A. The use of sodium azide and crystal violet in a selective medium for streptococci and Erysipelothrix rhusiopathiae / R.A. Packer // J Bacteriol - 1943. - V. 46, No. 4. - P. 343-349.

9. Seroprevalence of evolved selected pathogens in Iberian wild boar / M. Boadella, J.F. Ruiz-Fons, J. Vicente et al .//Emerg Dis. - 2012. - 59 (5). - P. 395-404.

10. Wang Q. Erysipelothrix rhusiopathiae / Q. Wang, B.J. Chang, T.V. Riley // J. Of Veterinary Microbiology. - 2010. - No. 140. - p. 405 -417.

11. Wood R.L. Handbook of Zoonoses / R.L. Wood, G.W. Beran // Erysipelothrix infections. - New York: CRC Press, 1994. - P. 83 - 91.

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