The inactivation of eggs of helminthes under the action of narrowband ultraviolet radiation of excilamps

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ABSTRACT

The inactivation of eggs of *Opisthorchis felineus* and *Diphyllobothrium latum* in the water under the action of UV excilamps at 222 and 282 nm in dependence on the surface dose of radiation was studied. It was observed that the water disinfection from eggs of helminthes was more efficient at 222 nm, than at 282 nm. At the surface dose up to 5 mJ/cm^2 of UV radiation at 222 nm up to 85 % of *Opisthorchis felineus* eggs were inactivated. At the comparable surface dose of UV radiation at 222 nm up to 56 % of *Diphyllobothrium latum* eggs were inactivated.

Keywords: Inactivation, helminth, disinfection, ultraviolet, excilamp

1. INTRODUCTION

In Siberia there are two rather wide-spread parasitic infections: opisthorchiasis (it's agent is *Opisthorchis felineus*) and diphyllobothriasis (it's agent is *Diphyllobothrium latum*). The agents of these diseases have three-stage life cycle. First the helminth's eggs infect fresh-water sellfish or zooplankton. The agents of the second stage are larvas - miracidium and procercoid. The second carriers are fresh water fish that contain the third stage agents (metacercarias and plerocercoids) in their muscles and internals. The final carriers are humans and predator mammals: cats, dogs, foxes, wolves and also pigs.

The prevention of these diseases can be performed on the first and third stages of the helminthes life cycle. The prevention of third stage is provided by proper cooking of fish and maintenance the hygiene. On the first stage it is necessary to prevent the infesting the water by helminth's eggs. It requires sterilizing the waste water. Nowadays sinkers, aeration and oxidative chemicals are used for dehelmintization. But not more than 75% of the eggs can be killed these ways[1], and there are permanent sources of helminthism.

The UV-radiation sterilization can become an additional method of waste water dehelmintization. The action of UV-radiation on the biological objects can be very different depending on wavelength and absorbed dose. The radiation in the range 320-400 nm (UV-A) is a part of solar radiation, living organisms have natural protection from this radiation. The spectral range 290-320 nm (UV-B) presents in solar radiation in spring and summer. Small doses of this radiation stimulate biological objects on pigmentation and useful substances synthesis. Big doses of UV-B radiation act negatively, lead to uncontrollable cell fission and damage DNA. UV-C is a radiation in the range 200-290 nm. On the sea-level this radiation is absent in all seasons. That's why living organisms don't have any protection from this radiation and even small doses have inactivating action on their development and reproduction.

Mercury lamps are widely used for disinfection and sterilization. Their radiation have line spectrum (see Fig.1), it can inactivate different bio-systems. But they contain mercury. When a lamp containing 80 mg of mercury is broken and the metal is fully vaporized the air is polluted[2] in the volume of 300000 m^3. So, in European Union it was decided to stop using mercury lamps step by step in medicine, biology and veterinary science.

The development of new UV-radiation sources – excilamps – changed their status from prototype equipment to production version[3,4]. Excilamps with KrBr, KrCl, XeBr and XeCl working molecules provides the radiation with different wavelengths that enables to make combined action on the objects in the range 206-308 nm[5]. The dose required to inactivate different types of viruses and bacteria is 5-20 mJ/cm^2.

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The excilamps have advantages in comparison with mercury lamps, also they don't contain mercury, that's why they are promising to be used in sterilizing devices[5-7].

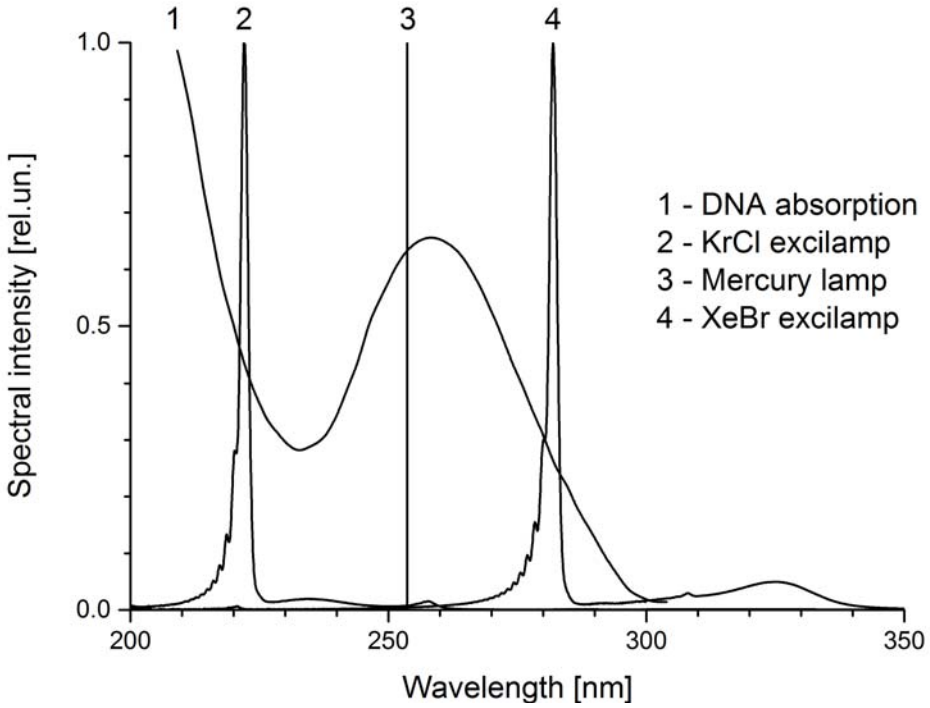

Figure 1. Absorption and emission spectra: 1 – general absorption spectrum of DNA, 2 − emission spectrum of barrier-discharge KrCl-excilamp with maximum at 222 nm; 3 – atomic line of mercury lamp at 253.7 нм; 4 − emission spectrum of barrier-discharge XeBr-excilamp with maximum at 282 nm.

In this work we summarize the first results on irradiating *Opisthorchis felineus* and *Diphyllobothrium latum* eggs in water for their inactivation by KrCl- and XeBr-excilamps radiation with spectral maximums at 222 and 282 nm respectively.

2. SAMPLES AND METHODS OF INVESTIGATION

The samples for research were prepared in Laboratory of parasitological investigations of Tomsk oblast Hygiene and Epidemiology center. Objects of research were helminthes eggs that were found in the probes.

The samples were prepared by the method of Kato thick swabbing[8]. The thin layer of fecal masses on the object carrier was covered by hygroscopic cellophane that was drenched by contact solution (glycerin, phenol 6% water solution and brilliant green 3% solution).

The experiment was made in the following way:

1. A sample was investigated by the microscopic method with the microscope MICMED-6 with photo-camera. The eggs were counted visually. On the Fig. 2 there are microphotos of *Opisthorchis felineus* eggs, made with ×10 zoom. The number of eggs in the sample was from 80 to 950.

2. The biomaterial with helminthes eggs was washed out from the object carrier by distilled water to the plastic container. Container was individual for every sample. The volume of water was 250-350 cm^3, the thickness of water layer was about 1-2 cm.

3. The container was irradiated through it's cover by KrCl- or XeBr-excilamp with the surface radiation dose 0.3-10 mJ/cm^2 and 4-100 mJ/cm^2 respectively. The irradiation time was from 15 to 120 seconds.

4. The irradiated liquid was poured into centrifuge test tubes and centrifuged for 5 minutes at 1000 rounds per minute. Supernatant fluid was removed.

5. The sediment was moved to the object carrier and investigated by the microscopy method for visual counting of the eggs and estimation of their morphology.

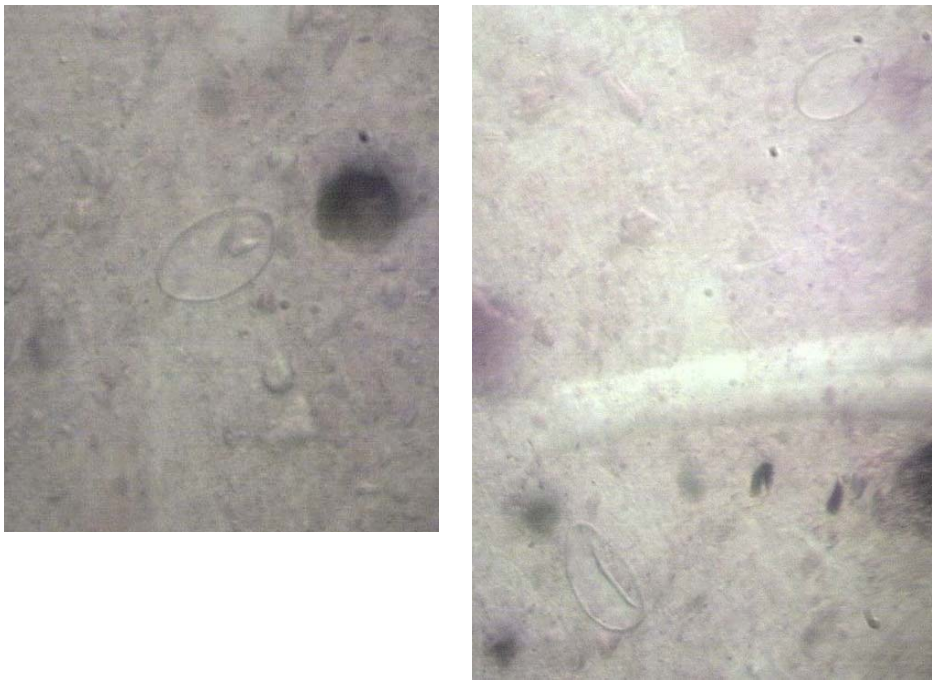

Figure 2. Microphotos of *Opisthorchis felineus* eggs. Magnification ×10.

Water samples infected by *Opisthorchis felineus* eggs were irradiated by KrCl- or XeBr-excilamps. Water samples infected by *Diphyllobothrium latum* eggs were irradiated only by KrCl-excilamp. Each sample was irradiated one time.

KrCl- and XeBr-excilamps developed in the Institute of High Current Electronics[4] were used for the irradiation of the samples. Their radiation spectra are on the Fig. 1 (λ_{KrCl} = 222 нм, λ_{XeBr} = 282 nm). The average radiation power was $P_{222} \cong 9$ mW/cm^2 and $P_{282} \cong 30$ mW/cm^2 on the surface of excilamp bulb. On the Fig. 3 you can see the dependence of the average power density from the distance to the lamp.

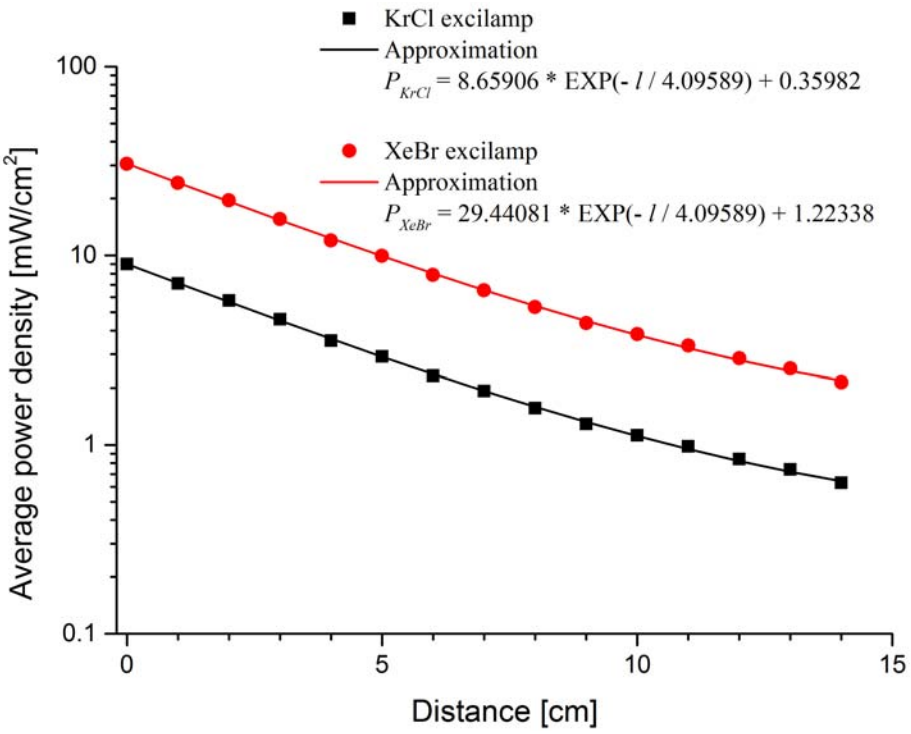

Figure 3. Dependences of average excilamp power density on the distance to the lamp. Black squares and curve – KrCl-excilmap. Red circles and curve – XeBr-excilmap.

3. RESULTS AND DISCUSSION

The results of our research are given on the Fig. 4. It is clearly seen that the inactivation of *Opisthorchis felineus* eggs by KrCl-excilamp radiation is more effective in comparison with XeBr-excilamp irradiation. The average power of KrCl-excilamp is in 3 times lower than that of XeBr-excilamp, but the percent of survived eggs after KrCl-excilamp irradiation is 40-70% less than after XeBr-excilamp action. The spectral maximum of KrCl-excilamp radiation ($\lambda = 222$ nm) corresponds to the photon energy of 5.6 eV, that is significantly than one for XeBr-excilamp ($\lambda = 282$ nm, photon energy is 4.4 eV). We assume that photons with bigger energy are better in destroying and embrittling the shell of the *Opisthorchis felineus* eggs. In our experiment from 15 to 30% of the eggs survived after 222 nm irradiation with the surface dose 5.1 and 0.3 mJ/cm^2, respectively. After 282 nm irradiation 70-90% eggs survived, the surface dose was 116-4 mJ/cm^2.

For one of the water samples with *Opisthorchis felineus* eggs we observed total absence of the eggs after 222 nm irradiation with surface dose 8.5 mJ/cm^2. Linear approximation of the survived *Opisthorchis felineus* eggs percentage after 222 nm irradiation enables us to estimate the value of the surface dose that is required for full inactivation – about 15.6 mJ/cm^2.

Irradiation of water samples with *Diphyllobothrium latum* eggs showed us smaller efficiency of 222 nm inactivation than for *Opisthorchis felineus* eggs samples. In our experiment 44-68% eggs survived after 222 nm irradiation with the surface dose 5.1-0.5 mJ/cm^2.

The differences in results for *Diphyllobothrium latum* and *Opisthorchis felineus* eggs can be connected with higher durability of *Diphyllobothrium latum* eggs shell and differences in the experimental conditions, such as volume of water, it's turbidity and eggs distribution.

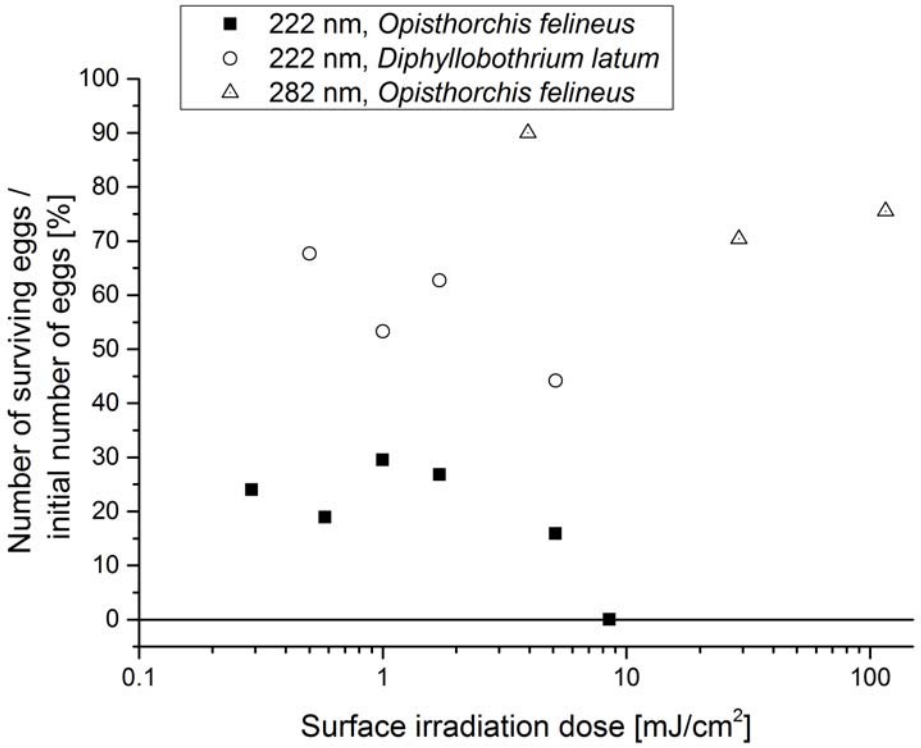

Figure 4. Dependences of ratios of amount of *Opisthorchis felineus* and *Diphyllobothrium latum* eggs after irradiation to eggs amount before irradiation on surface radiation dose of KrCl- and XeBr-excilamps.

4. CONCLUSION

In this work we present the first results of the experiments on the disinfection of the water containing *Opisthorchis felineus* and *Diphyllobothrium latum* eggs by the UV-radiation of the excilamps with the spectral maximums at 222 and 282 nm, respectively.

The level of inactivation was 40-70% higher after 222 nm irradiation than after 282 nm irradiation. After UV-irradiation of the water containing *Opisthorchis felineus* eggs with the surface dose 5.1-0.3 mJ/cm^2 only 15-30% eggs survived.

The level of *Diphyllobothrium latum* eggs inactivation after UV-irradiation at 222 nm was significantly lower than one for *Opisthorchis felineus* eggs under the similar conditions. With the surface dose 5.1-0.5 mJ/cm^2 44-68% of *Diphyllobothrium latum* eggs survived. The differences in the results for different helminthes can be explained by the influence of different distribution of the eggs in the container, their non-homogeneous irradiation and different turbidity of the water in the samples. The systematical researches with the identical experimental conditions are needed to obtain thereliable dependence of the survived eggs from the surface dose. Also we cannot exclude the possibility that the eggs, visually detected by microscopy after irradiation, could be dead. On the other hand, their shell can contain pigment (e.g. bilirubin) that is able to decrease the efficiency of UV-irradiation.

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