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**ISOLATION OF OPISTHORCHIS FELINEUS METACERCARIAE BY
EXPERIMENTAL INFECTION OF THE GOLDEN HAMSTER MESOCRICETUS
AURATUS IN THE LABORATORY**

Abstract

Today, there are natural foci of many dangerous parasitic diseases on the territory of Kazakhstan, which requires the veterinary service to take effective measures to prevent, monitor, diagnose and treat, as well as to prevent the spread of these diseases to other regions of our country.

Currently, the proportion of fish and fish products in the human diet has increased, which increased the risk of people becoming infected with dangerous invasive diseases, among which opisthorchosis is of the greatest importance in human pathology. The difficult epizootic situation and the insufficient information content of the methods used to identify the causative agent of this disease led to the conduct of scientific research.

According to the statistics of the authorized body in the field of epidemiological surveillance of the Republic of Kazakhstan, up to 1,500 cases of opisthorchiasis are recorded annually in the republic. This circumstance is directly related to food security, because the source of the invasion is river fish, which is widely represented in the diet of the local population. The incidence rate of the population in opisthorchosis in the West Kazakhstan region is 3 times higher than the republican figure and is 21.7 per 100 thousand population. The source of opisthorchiasis invasion for humans, domestic and wild carnivores is the fish of the carp family (Cyprinidae), which are widely distributed in the region's water bodies and has an important epidemiological and epizootological significance.

At the present stage of laboratory diagnostics for the detection of fish opisthorchiasis, muscle samples with subcutaneous tissue are examined by a compression method, which in turn is subjective, and the parasite's identification fully depends on the experience and competence of the researcher, interpretation of the result is completely subject to the human factor.

In order to obtain the mature form of *O.felineus*, a bioassay on a golden hamster was carried out, since this laboratory animal is susceptible to various diseases, including opisthorchiasis.

Keywords: *Mesocricetus auratus*, *opisthorchosis*, *Opisthorchis felineus*, *Cyprinidae*, *diagnostics*, *experimental infection*.

Introduction. Opisthorchiasis is an invasive disease of dogs, cats, foxes, Arctic foxes, sables, martens and other carnivores, as well as pigs and humans, caused by the trematode *Opisthorchis felineus*, parasitic in the bile ducts of the liver, pancreas and gall bladder.

According to Bronstein A. trematodes of the liver belonging to the family Opisthorchiidae affected about 17 million people, and about 350 million people living in 13 countries of the world are at risk of infection.

Fish of the carp family are additional hosts and the main source of infection of humans and animals by opisthorchiasis. The final hosts of opisthorchis, in whose bodies mature forms of helminth develop, are domestic and wild carnivores, as well as humans [1, 2].

Thus, helminths develop with the participation of an intermediate host - freshwater mollusk (bitiny), an additional - freshwater fish of the carp family (ide, dace, rudd, bream, crucian carp, tench, minnow, roach, etc.) and definitive - domestic and wild carnivores (mostly dogs and cats) and humans [3].

The cercariae are located, as is well known, in the musculature of fish, mainly in the superficial layers of the back, less often in fins, gills; then cercariae develop into metacercariae, which are invasive stages that can infect humans and carnivores [4].

Human cases of opisthorchosis have been reported in 9 administrative territories. Every year in Kazakhstan there are more than 1,300 cases of opisthorchiasis, the incidence rate is 7,0–8,0 per 100 thousand population. The highest incidence of people with this invasion was noted in the West Kazakhstan, Pavlodar and Akmola regions, where the incidence rate reaches 113,2 per 100 thousand population.

The goal was to experimental infection of golden hamster *Mesocricetus auratus* with metacercariae *Opisthorchis felinus* and pick out the helminths from liver.

Materials and research methods. The research works were conducted in the laboratory of biotechnology engineering profile of the science management of the Zhangir Khan West Kazakhstan Agrarian Technical University.

In order to conduct research and identify the causative agent of opisthorchiasis *O.felinus*, fish were harvested in the rivers Derkul and Utva. A total of 20 specimens of carp fish were researched (ide - 4, rudd - 4, crucian - 12), as they are potential vehicle of *O.felinus* larvae.

Research results. A compression method was used to detect metacercariae in fish tissue. More accurate results were obtained in the researching of the entire surface of the muscle tissue of fish, which was carefully separated along with subcutaneous fat from the skin and fins. For a compression study, in order to save time, 5 muscle samples with subcutaneous tissue on both sides of the fish were examined: 3 samples from the dorsal and 2 from the ventral part of the muscles on each side. Each muscle sample was taken from an area of 1-2 cm² at a depth of 2-3mm (table 1) [5].

Table 1 - Infection of fish in the carp family of opisthorchiasis metacercariae

Indicators	Derkul river	Utva river
Kind of fish	ide = 2 rudd = 2 crucian carp = 6	ide = 2 rudd = 2 crucian carp = 6
Researched, sample	10	10
Infected, sample	0	10
extensiveness of invasion, %	0	100
intensity of invasion, sample, from-to	0	1-36

As can be seen from the table, the vehicle of opisthorchiasis are fish from the river Utva. The following indicators were obtained: the extensiveness of invasion (EI)– the relative infection rate of

fish and the intensity of invasion (II) —the indicator of the number of metacercariae in one fish. The remaining fish species were free from opisthorchiasis larvae [6, 7].

Then, after the detection of invasive helminths *O.felineus*, we began the experimental part of our research work. The golden hamster was fed opistorhis with fish, food and water. To do this, the crushed muscles of the affected fish were laid in carrots with a core removed. When working with a hamster, the rules of personal prevention were followed.

On the 14th day of the experiment, clinical signs of opisthorchiasis began to be expressed: lethargy, partial baldness, body temperature rise of 1.5-2⁰C, diarrhea. There is an increase in the abdominal cavity and the yellowness of the mucous membranes. The hamster is constantly in a sad state, sitting motionless or sleeping. On palpation of the abdominal cavity an enlarged liver is detected. Diarrhea gives way to constipation (figure 1).

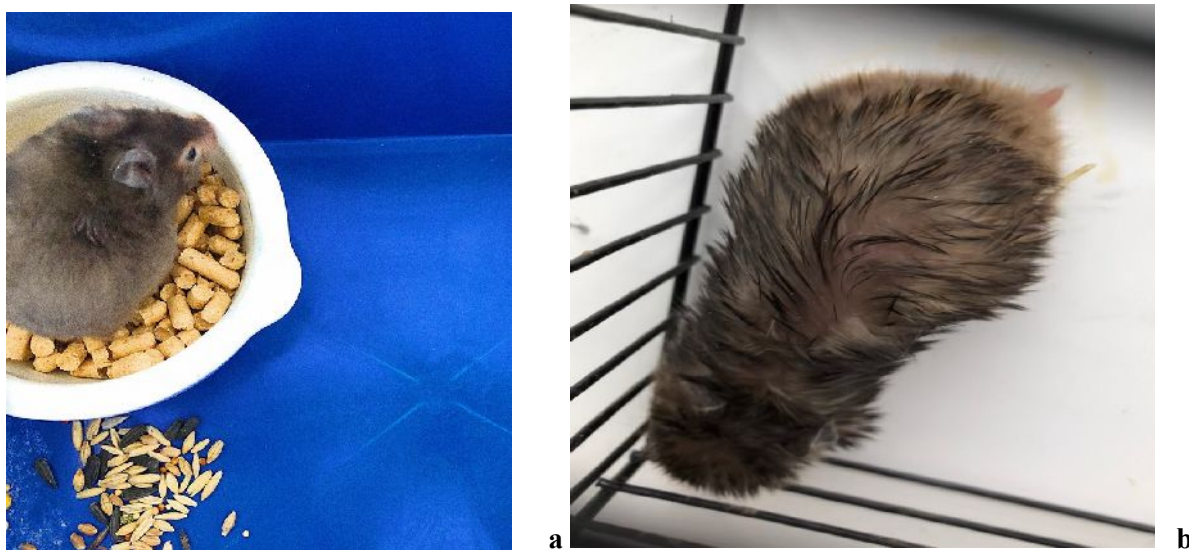


Figure 1 – Hamster before infection with opisthorchiasis (a), on the 14th day of the experiment (b)

On the 20th day of experimental infection, a hamster was dissected, and pathological changes in the organs were revealed, including hemorrhagic extravasation in the abdominal cavity, an enlarged and hyperemic liver, and invasive helminths *O.felineus* were also detected (figure 2).



Figure 2 - Enlarged and hyperemic liver of the golden hamster

Opisthorchis felinus is a parasite that has a flat elongated body, narrowed at the front, rounded at the back. Its length is from 5.8 to 18 mm, width from 0.75 to 2.2 mm. On its ventral side there are suckers of almost the same size - oral and abdominal. From the oral sucker departs the pharynx, which then passes into the esophagus and into the two branches of the intestine, which blindly terminate behind the posterior testicle.

Like most trematodes, opisthorchid is hermaphroditic. Male reproductive organs are represented by two lobe testes, located in the back of the body, by seed tubes and a cirrus bursa. The female reproductive organs are represented by a single ovary, located in front of the testes in the posterior half of the body, the turbulent uterus, which is filled with ripening eggs and fills the entire middle of the body (figure 3).



Figure 3 – Metacercaries isolated from hamster’s liver

Conclusion. The research work found that the localization site of *O.felineus* is located in the floodplain of the river Utva, while the extensiveness of fish invasion was 100% and the intensity of invasion ranged from 1 to 36. The causative agent of the disease *O.felineus* is located in the muscles of the fish, mainly in the surface back layers. The disease is dangerous for both carnivores and humans. Therefore, the study of this disease to this day remains relevant.

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ТҮЙІН

Қазіргі таңда Республика территориясында көптеген жұқпалы және инвазиялық аурулардың ошақтары белгілі. Бұл жағдай ветеринарлық қызметтің тыңғылықты жұмысын талап етеді, соның ішінде: аурулардың алдын алу, мониторинг, балау, сонымен қатар, аталмыш аурулардың еліміздің басқа аудандарына тарап кетуін болдырмау мақсатында жүргізілетін шаралар.

Соңғы кезде адамдардың тамақтану рационында балық және балықтан жасалған өнімдердің үлесі әлдеқайда жоғары, бұл олардың аса қауіпті инвазиялық ауруларды, соның ішінде описторхозды жұқтыру қаупін одан әрі жоғарылата түседі. Еліміздегі эпизоотиялық жағдай, аталмыш аурудың қоздырғышын анықтауда және оны басқа ауру қоздырғыштарынан ажыратып балауда қолданылатын тәсілдердің жеткіліксіз деңгейде мағлұмат беруі ғылыми зерттеу жүргізуге түрткі болды.

Қазақстан Республикасының эпидемиялық қадағалау қызметінің статистикасына сүйенсек, республикамызда жыл сайын описторхозды жұқтырудың 1500-ге жуық жағдайы тіркеледі. Аталмыш жағдай өндірістік қауіпсіздікпен тікелей байланысты, өйткені, инвазияның көзі жергілікті халық жиі тұтынатын өзен балығы болып табылады. Батыс Қазақстан облысы бойынша описторхозды жұқтыру көрсеткіші республикалық көрсеткіштен 3 есе асып түсіп, 100 мың адам басына шаққанда 21,7-ні құрайды. Адамдар, үй және жабайы жануарлар описторхозы инвазиясының көзі тұқы тектес балық (*Cyprinidae*) болып саналады, аталып отырған балық түрі облыстағы су қоймаларында кең таралған және маңызды эпидемиологиялық және эпизоотологиялық маңызға ие.

Заманауи зертханалық балау кезінде описторхозды анықтауда балықтың тері асты клетчаткасында орналасқан бұлшық етті компрессиялық әдіспен зерттейді, бұл өз кезегінде субъективті баға беру болып табылады. Ал паразитті дұрыс ажыратып балау зерттеушінің тәжірибесі мен біліміне, яғни алынатын нәтиже толықтай адами факторға тікелей байланысты.

Зерттеу жұмысы барысында *O.felineus* қоздырғышының ересек формасын бөліп алу мақсатында *Mesocricetus auratus* аламанына биологиялық сынама қойылды. Өйткені бұл зертханалық жануар көптеген жұқпалы ауруларға, соның ішінде описторхозға аса сезімтал келеді.

РЕЗЮМЕ

На сегодняшний день на территории Казахстана существуют природные очаги многих опасных паразитарных заболеваний, что требует от ветеринарной службы эффективных мер по профилактике, мониторингу, диагностике и лечению, а также по предупреждению распространения этих болезней в другие в другие регионы нашей страны.

В настоящее время в рационе питания человека возросла доля рыбы и рыбопродуктов, что повысило риск заражения людей опасными инвазионными заболеваниями, среди которых наибольшее значение в патологии человека занимает описторхоз. Сложная эпизоотическая ситуация и недостаточная информативность используемых методов идентификации возбудителя данного заболевания сподвигли на проведение научного исследования.

Согласно статистическим данным уполномоченного органа в области эпидемиологического надзора Республики Казахстан ежегодно в республике регистрируется до 1500 случаев заболеваемости описторхозом. Данное обстоятельство напрямую связано с продовольственной безопасностью, т.к. источником инвазии является речная рыба, достаточно широко представленная в рационе питания местного населения. Уровень заболеваемости населения по описторхозу в Западно-Казахстанской области в 3 раза превышает республиканский показатель и составляет 21,7 на 100 тыс. населения. Источником инвазии описторхоза для человека, домашних и диких плотоядных является рыба семейства карповых (*Cyprinidae*), которые широко распространены в водоемах области и имеет важное эпидемиологическое и эпизоотологическое значение.

На современном этапе лабораторной диагностики для выявления описторхоза рыб проводят исследование проб мышц с подкожной клетчаткой компрессионным методом, что в свою очередь является субъективным, а идентификация паразита полностью зависит от опыта и компетентности исследователя, т.е. интерпретация результата полностью подтверждена человеческому фактору.

В целях получения половозрелой формы *O.felineus* была проведена биопроба на золотистом хомячке, так как данное лабораторное животное восприимчиво к различным заболеваниям, в том числе к описторхозу.