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Role of Birds and Invertebrates in Epidemiology and Epizootology of Trichinellosis at Chukotka Seashores

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Abstract: A laboratory model of circulation of the Arctic nematodes *Trichinella nativa* passing through birds' intestines has been developed. Infective larvae of the *Trichinella* Arctic strain isolated from muscles of a draught dog in Lorino settlement, Chukotka Autonomous District, Russian Federation, were used throughout the experiment. White mice, golden hamsters, quails, and various species of gulls were experimentally infected with the larvae mentioned. Later some infective larvae from quail faeces were fed to mammals and birds, as well as to various invertebrates: dipteran larvae, mollusks, crustaceans. It is established that faeces of experimentally infected birds are not only dangerous to mammals, but at the same time facilitate transition and accumulation of infection through transport hosts – various invertebrates. These invertebrates are important in trophic connections of many mammals and certainly play an important part in functioning of the parasitary systems formed by *Trichinella* in both water and land biocenoses.

Key words: *Trichinella*, role of birds and invertebrates, epidemiology, epizootology, Chukotka seaboard

Introduction

At Arctic conditions there is a connection between trichinellosis outbreaks among natives of the Arctic seashores and usage of the sea mammals' meat. According to statistics, the rate of infection with *Trichinella* of walruses (*Odobenus rosmarus rosmarus* and *Odobenus r. divergens*) is from 1 to 4%, and that of seals and sea lions (*Erignathus barbatus*, *Phoca hispida, Phoca groenlandica, Eumetopias jubatus*) is from 0.8 to 1.2 % (Margolis *et al.* 1979, Moller *et al.* 2005, Mclntyre *et al.* 2007). The infection rate of polar bears (*Ursus maritimus* L.) feeding on young walruses and seals reaches 50% (Forbes 2000, Leclair *et al.* 2004, Rausch *et al.* 2007). However, the question of paths of infection with *Trichinella* in sea mammals remains unanswered. An analysis of trophic connections between these mammals shows that *Phoca* spp. mainly feed on fish and invertebrates, especially crustaceans (crabs, shrimp, amphipods, mysids).

The basis of diet in bearded seals consists of sea floor invertebrates (crabs, shrimps, hammarids), gastropods and cephalopods, benthic fishes, etc. Only walruses, although mainly feeding on sea floor bivalves, may sometimes attack seals or eat carcasses of vertebrates.

Abs, Schmidt (1954) quoted by Britov (1962), formulated a theory about a role of birds, in particular, gulls (*Gavia alba* Gunn*., Fulmarus glacialis, Pagophila eburnea* Phipps), in distribution of trichinellosis in the High Arctic. The authors supposed that gulls that feed on the corpses of animals infected with *T. nativa,* may swallow its larvae and later spread them through faeces in environment. The authors also assume that infected bird faeces may be a source of infection for sea mammals, which theory has not been experimentally proven by now.

The goal of the present work was to experimentally prove the possibility of mechanical transmission of *Trichinella* spp. larvae through bird faeces to land and sea mammals, with invertebrates (dipteran larvae, mollusks, crustaceans) also playing a part.

Materials and Methods

Materials: Infective larvae of an Arctic isolate of *T. nativa* (3rd passing) initially obtained from muscles of a draught dog (*Canis familiaris* L.) from Lorino settlement, Chukotka Autonomous Distinct, Russian Federation were used in the work.

Animals used: white mice (*Mus musculus musculus* L.) – 20; two-month-old quails (*Coturnix coturnix* sp.) – 50; hamsters *Mesocricetus auratus* (Waterhouse, 1839) – 30; mollusks *Buccinum bryani* and *Natica clause* – 18 each, larvae of *Chironomus plumosus* – 200, sea shrimps *Sclerocrangon boreas* - 127, fly larvae (order Diptera, fam. Calliphoridae*,* species *Calliphora walensis*) – 139, 2 gulls *Larus glaucescens* and 1 gull *Larus hyperboreus*.

Methods: Infective larvae from muscles of naturally infected draught dog were obtained through digestion of samples in artificial gastric fluid. Infection of young hamsters *per os* was performed using 2 larvae per 1 g of body mass. The infective stage had been reached after 30-45 days, after which the hamsters were killed with diethyl ether, skinned and intestinal organs removed. Each carcass was cut in half lengthwise. The first half was weighted, minced, and then digested in artificial gastric fluid in order to find the precise number of larvae in 1 g of bone-muscle mass. According to our data, a mass of minced meat needed to infect 1 quail in free feeding taking 800-1000 larvae per bird was determined. The faeces from infected quail were obtained each 12 hours for 2 days. To prevent drying off of the faeces, a tray with some water was placed under each wire cage. The content of the trays were taken to gauze bags, placed into chemical glasses, and then filled with saline and left for 2 hours at 38°C. During that period all the viable *Trichinella* from the faeces precipitated to the glass's bottom while the immobile and deformed specimens remained in the fecal mass, their numbers being determined after application of a method for repeated rinsing. All subsequent experiments used quail faeces containing large numbers of living decapsulated *Trichinella*. Experiments were performed to determine possibilities of infection with decapsulated larvae expelled into water or moist environment with birds' faeces in following animals: mollusks, dipteran larvae, crustaceans (shrimps). Degrees of involvement of these animals – transport hosts of *Trichinella* – in trophic connections with mammals, as well as their role in functioning of *Trichinella* parasitary system were assessed. The infectivity of *Trichinella* larvae placed into intestine of these species was determined by using biological tests on hamsters each of which was infected with 50 randomly selected larvae. The hamsters' intestines, muscles, and blood were then assessed using conventional methods for presence of adult, migrating and larval forms of *Trichinella*. Each experiment was repeated at least 3 times.

Results

Studies of infectivity and viability of *T. nativa* larvae passed through birds' digestive tract

At first day after infection there were large numbers of living decapsulated *Trichinella* in the quails' faeces. The birds' faeces were liquid, covered with dense mucus, sometimes with blood streaks; however, there were neither depression nor loss of appetite in the quails. During first day the infected quails had excreted about 60% of introduced *Trichinella*, with all parasites being decapsulated (Fig. 1) and highly mobile. *Trichinella* rinsed from faeces were used to infect five hamsters *per os* (50 larvae per hamster). At second day numbers of expelled parasites dropped and there were many more untwisted larvae with loss of their structure (about 15-20% from the total numbers of those rinsed from the faeces). These larvae were rinsed again and used to infect 3 hamsters by the same dosage. The examination of the muscles in both experimental hamster groups showed that all the animals had been infected with trichinellosis, with different intensity of infection (from 1.2 to18.6 larvae/g).

After sacrificing and examining 2 birds on the third day after infection, it was found that all sections of their intestine contained male and female *Trichinella* in ratio of 1:2, with their sexual systems already formed. Lengths of females were 1.6-2.25 mm; those of males were 0.95-1.15 mm. Many *Trichinella* from the mucous layer of the intestine demonstrated an original form of head end, like a strongly bended hook. All obtained helminths were quite active. Most

females contained eggs in their ovaries, closely connected cells with large nuclei.

At the 5th and 7th days after infection, no *Trichinella* larvae were found in the quails' faeces. After the sacrifices of the text animals, a large number of adult males and females, in ratio of 1:3, were found in the intestines, with practically all the females containing larvae at different development stages, and many of them having the forepart of uterus filled with larvae (Fig. 2a). Lengths of females reached 2.75-3.15 mm, while those of males (Fig. 2b) were 1.0-1.3 mm.

After the sacrifices at the 9th and 14th days some decrease in total number of the helminths in the quails' intestines was found, but the uteri of all the females were filled with embryos and larvae, some of the larvae already leaving the females. The sizes of newborn larvae were 90-110 μm. We did not found young larvae in the quails' blood or muscles, which may be a result of small size of the objects and difficulty in their identification. Dissections of the quails' intestines at the 21st day after infection showed one single adult *Trichinella* in each of only 2 birds out of 6. The examination through compressor trichinelloscopy of the skeletal muscles after their digestion in artificial gastric fluid had produced negative results.

Thirty days after the infection there were no *Trichinella* found in the intestines, blood, or skeletal muscles of the infected quails.

Studies on the viability of *T. nativa* larvae excreted by the quails during two first days after infection and spending various times in moist environment at room temperature showed that *Trichinella* had not lost its infection ability under these conditions. The larvae were obtained from the birds' faeces and rinsed with warm saline several times. The living and dead helminths were counted; then the level of morphological changes was assessed and a biological test was performed on mice. After 35 days the mice carcasses were studied using a method of digestion in artificial gastric fluid. All rodents infected with *T. nativa* larvae from quails' faeces after 5, 10, 14 days of incubation in tubes at room temperature, showed *Trichinella* infection with varying intensity. Only one mouse of three was found infested after 3-week exposure to *Trichinella* larvae.

Studies of role of invertebrates in transmission of trichinellosis to mammals through bird faeces

Role of chironomid larvae

Larvae of mosquitoes of order Diptera, family Culicidae, genera *Anopheles, Culex, Aedes,* etc., and their relatives are an important component of continental water bodies' benthos and a main source

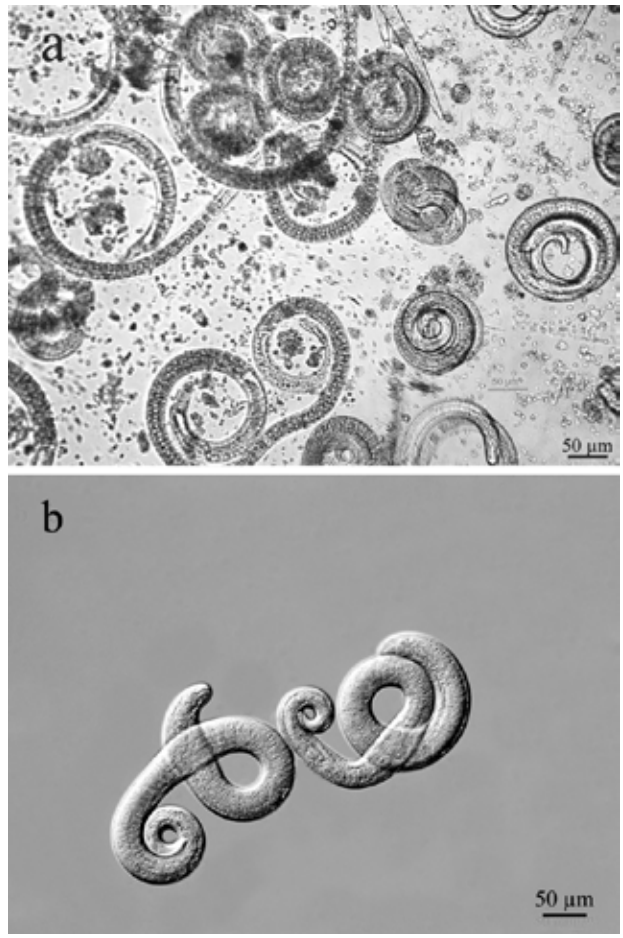

Fig. 1. Decapsulated *Trichinella* larvae from quails' faeces, 24 hours p.i. a. Bright field microscopy; b. Differential interference contrast

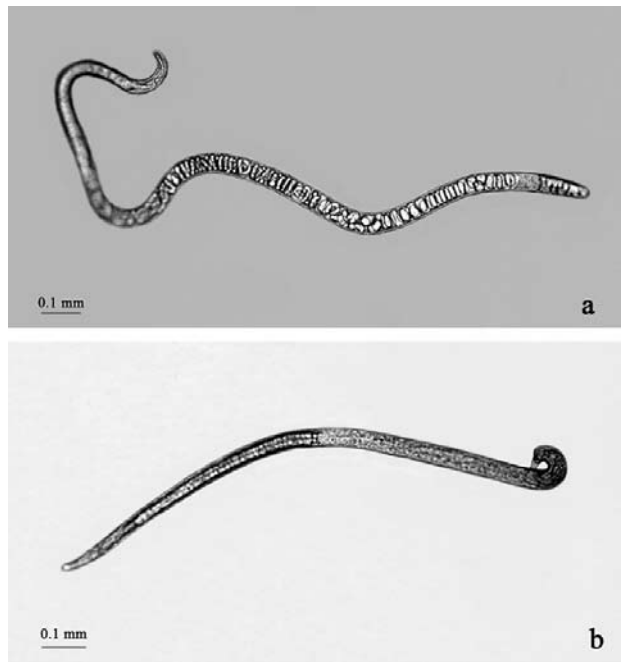

Fig. 2. Mature *Trichinella* in quail intestine at 7th day p.i.: a. Female; b. Male

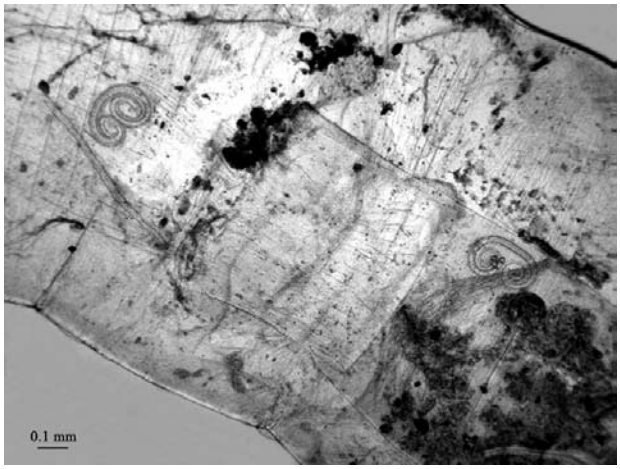

Fig. 3. *Trichinella* larvae in bloodworm digestive system

of food for fishes, water birds and other animals.

Larvae of *Chironomus plumosus* from family Chironomidae were used in the first experiment. We used the faeces of quails fed with minced meat containing encapsulated larvae of an Arctic isolate of *T. nativa* for a day. The decapsulated larvae rinsed many times with tap water were placed into Petri dishes, 1000 into each, where 20-25 chironomid larvae were then placed. In a control thoroughly minced meat containing encapsulated *Trichinella* larvae in the same number was used. The process of swallowing decapsulated *Trichinella* in the experiment or meat fragments containing encapsulated ones in the control by the chironomid larvae was studied under a binocular microscope at 2x8 magnification. On that examination no swallowing of muscle fragments with encapsulated *Trichinella* by the chironomid larvae was observed, which may be attributed to the incompatibility of the larval oral apparatus with the offered food. The presence and distribution of *Trichinella* along the chironomid larvae digestive systems were studied using compressor method preparations (Fig. 3). It was established that the chironomid larvae had swallowed easily the decapsulated *Trichinella* and the latter were passed through the chironomid digestive system practically undamaged. To confirm the *Trichinella* infectivity, the bloodworms were extracted from the Petri dishes with pincers, carefully rinsed in clear water, mixed with pounded sunflower seeds and fed to mice and hamsters. The result of this test was positive - all animals were found infected with *Trichinella*.

Role of fly larvae

Larvae of *Calliphora walensis* (order Diptera, family Calliphoridae) were used in these experiments. To attract the flies under city conditions, hermetically sealed plastic containers were placed in open air and filled with food remains. Holes of 0.8 cm in diameter were made in the covers of the containers. When adult flies were seen on the substrate, the holes were covered with cotton swabs, the containers transferred into the laboratory, and the development of the larvae was observed for 3-4 days. After that, the larvae, 67 in total, and with a mean length of 0.5, were transferred into a Petri dish onto an organic substrate mainly consisting of faeces of quails fed with *Trichinella*-infested meat, with small amounts of compound feed and water. After the larvae had spent one day on the infested medium (and the latter had transformed into a viscous semi-liquid mass), the content of their intestines was studied. For that purpose, their intestines were separated from cuticle, fat body and trachea using 2 dissecting needles under binocular microscope, then rinsed in 50% glycerin, after which the compressor trichinelloscopy was performed. In these studies, the number of *Trichinella* larvae in various sections of the intestines, shapes of their bodies and the presence of structural changes were noted.

The results showed that linear, often fragmented *Trichinella*, mainly with damaged cuticle and internal organs, were present only in the anterior section of the intestine. Final enzymatic degradation of the larvae took place while they passing through the intestine, so no fragments of them were observed in the posterior gut. Probably, the influence of extra-intestine digestion by flies' larvae on already decapsulated *Trichinella* expelled from birds with faeces is deadly for these helminths.

Similar results were obtained during experiments at the Chukchi and Bering seashores (Fig. 4). The larvae of flies (72 in total), with lengths of 0.8-1 cm, were obtained from rotting wastes of the sea fishery on a dump near Lorino. Some glaucous-winged gulls (*Larus glaucescens*) and one glaucous gull (*Larus hyperboreus*) were caught there and placed in separate cages. During the experiment these birds were fed with large amounts of *Trichinella*-infested meat (the intensity of infection with *T. nativa* being 24.7 larvae/g) of captive polar foxes (*Alopex lagopus*). The bird faeces containing living, decapsulated *Trichinella* were used as an organic substrate for feeding the blue carrion fly larvae. After 24 hours the larvae were rinsed in running water and investigated for presence of viable *Trichinella* in their intestines. A total of 8 fragments of dead *Trichinella* were found, with linear shape and clear signs of damage to cuticle and internal organs. Thus, the role of carrion fly larvae in transit of *Trichinella* through birds' faeces to mammals was found to be only an elimination of infective agent and completely precludes such path of infection in mammals.

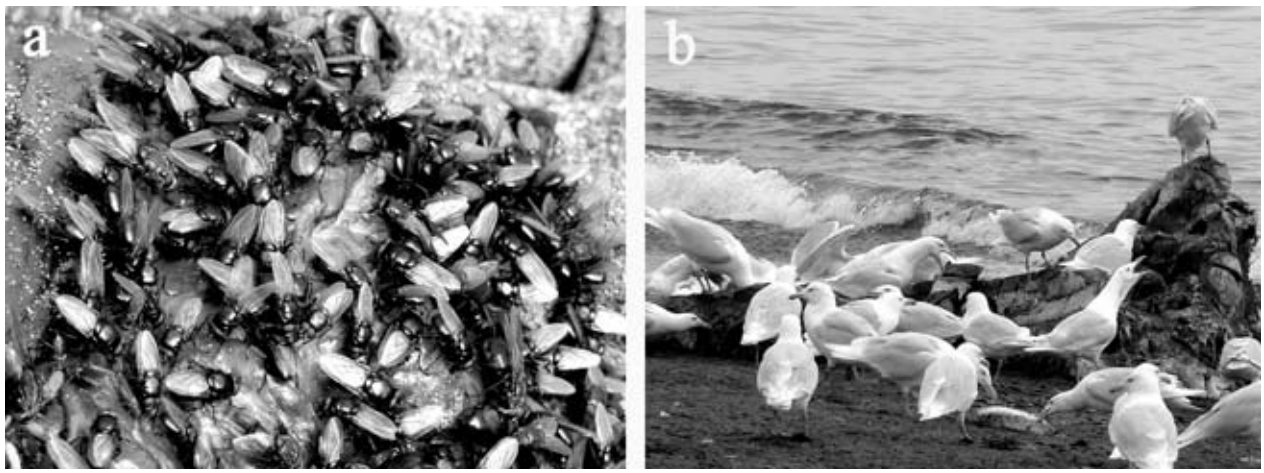

Fig. 4. Chukotka Autonomous District: a. Blue carrion flies on thrown wastes of sea fishery (cutting ground, Lorino settlement); b. Sea-gulls on the whale remains

Role of mollusks

The gastropods *Buccinum bryani* and *Natica clause* from the Bering Sea were used to define the possible role of mollusks in distribution of *Trichinella* in the basins of the Arctic seas. The mollusks were placed into an aquarium with seawater and after two days of fasting were fed with decapsulated *Trichinella* larvae rinsed from the faeces of the gulls that had been fed with minced meat infested with *T. nativa* larvae during the previous day. After 24 and 48 hours, respectively, the mollusks were taken from water, rinsed with tap water and moved into a clear Petri dish with small amount of seawater. Then the mollusk faeces and the content of their intestines were studied using compressor microscopy.

At 24th hour after the infection, the faeces of the mollusks from the experimental group were found to contain linear *Trichinella* larvae (Fig. 5a). Forty-eight hours after the infection the faeces contained larvae only in small numbers and with degraded structure. A considerable number of both straightened almost linear *Trichinella* larvae and shaped as non-compact spiral or comma larvae were observed in the snail digestive system. In the faeces and digestive system of snails that had been fed on the muscle tissues with encapsulated *Trichinella*, some single helminths, being mainly encapsulated, were registered. White inbred mice infected *per os* with 50 *Trichinella* isolated from the snail faeces, were used as bio-tests. To study a possibility of mammal infection with *Trichinella* through feeding on living mollusks, golden hamsters were fed with the latter, 5 mollusks each. As a result, 8 hamsters out of 10 were infected. The encapsulated *Trichinella* larvae in hamster muscles at the 90th day after the infection with the experimentally infected snails can be seen on Fig. 5b. The results show that, while being in the water, the snails found and actively consumed decapsulated *Trichinella* extracted with the bird faeces. No development of adult *Trichinella*

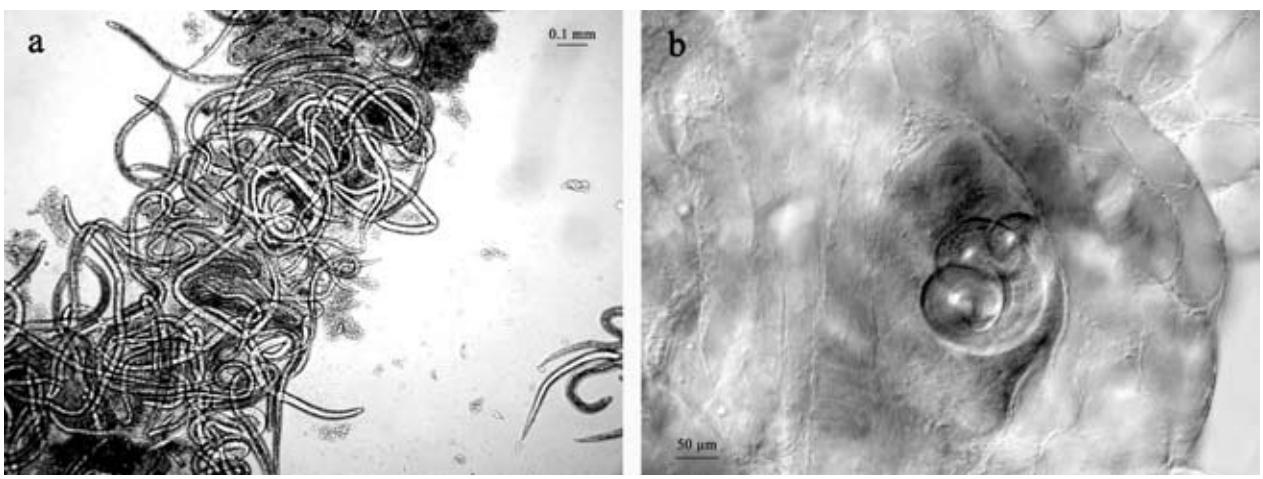

Fig. 5. *Trichinella* larvae: a. In faeces of experimentally infected snails; b. *T. nativa* infective larvae in hamster muscles

was observed in the snail intestine, with the majority of helminths expelled with faeces remaining vital and infective.

Role of crustaceans

An analogous experiment was performed using the sea shrimp *Sclerocrangon boreas* (order Decapoda) from the Bering Sea. The results showed that the shrimp could actively collect and consume the *Trichinella* larvae from the bottom of the aquarium. On studying by the method of the compressor trichinelloscopy, a sufficient number of *Trichinella* larvae and fragments were observed in the prepared shrimp intestine at 6th, 12th and 24th hour. Feeding white the mice and hamsters on both shrimp and their faeces (mixed with sunflower seeds) resulted in infection for all the experimental animals.

Discussion

The results of the present studies showed that quails easily survive dosages of infective *Trichinella* larvae, which would be lethal to mammals. This is probably owing to the secretion of large amounts of mucus by the intestine cells in response to the infestation, which causes quick expelling of most helminths. *Trichinella* expelled into the environment remain infective for a long time, allowing the mechanical transmission of trichinellosis from birds to invertebrates, and thereafter to sea and terrestrial mammals.

Large gatherings of birds at the shores and islands of the Arctic seas in summer and their manifold trophic connections, including pecking at carcasses of land and sea mammals, may indirectly affect the *Trichinella* circulation among the sea mammals. The bird excrements containing *Trichinella* are dissipated in the water and used as food by fish, crustaceans, mollusks, various plankton invertebrates, which are, in their turn, the main food source for various whales and pinnipeds of the Arctic seas. It is known that the main elements in the *Trichinella* circulation are carcasses of land and sea mammals, and the wastes of the sea hunting, which are thrown into the sea by the natives, but such mechanical vectors as birds and invertebrates are also contributing to the distribution of *Trichinella* throughout the Arctic territories.

The present laboratory study clarifies many questions of the epizootology of trichinellosis in the basins of the Arctic seas. The sea mammals are infected by the *Trichinella* spread in the water through the remains of the dead animals, through the faeces of the carnivorous birds, via the intestines of which most infective larvae are transited, and through trophic connections with a wide range of poikilothermic sea animals. Our results support a the hypothesis of Abs, Schmidt (1954) quoted by Britov (1962), that birds are mechanical vectors of this infection. The faeces of birds that have consumed *Trichinella*-infected meat not only constitute an infectious hazard to mammals, but also facilitate transmission and accumulation of the infection to the transport hosts of *Trichinella*: mollusks, crustaceans, dipteran larvae, etc. These invertebrates rank high in trophic connections of many mammals and are clearly important for the functioning of the *Trichinella* parasitary system. The experiments performed show a biological possibility of infections of mammals with *Trichinella* via the above-mentioned invertebrates - "passive" vectors of this infection.

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