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Results of scientific expedition to natural biotopes of the Republic of Tyva in 2019 with the purpose of infectious disease monitoring in wild bird populations

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SUMMARY

The results of the scientific expedition to Tere Khol and Uvs Nuur Lakes in the Republic of Tyva with the purpose of active monitoring of highly dangerous diseases in wild migratory waterfowl and epidemic analysis of these biotope water areas are presented in the paper. The Uvs Nuur Lake is a kind of an indicator for avian influenza introduction to the Russian Federation, because this is the resting and nesting area for many migratory wild birds during the period of mass migrations from Central and South-East Asian countries. In the process of active monitoring the complete autopsy of bird carcasses with description of organs and systems and sampling for laboratory diagnostics were performed. Droppings (pooled samples), parts of internal organs from dead and shot birds, blood (if possible) served as biological and pathological material for testing. While sampling, species were identified using an ornithological guide. The autopsy of dead waterfowl and birds shot for diagnostic purposes demonstrated a high worm burden of nematodes and cestodes. Two samples from European herring gulls were positive for avian influenza type A virus genome and subtype H13N6 was identified in one of them. Avian paramyxovirus serotype 1 (APMV-1), the agent of Newcastle disease, was found in one sample from gulls. The lakes of the Republic of Tyva are the most significant sites for sampling of biological material from wild birds, as the primary detection of highly pathogenic avian influenza virus in this territory is a serious signal of potential further virus spread and a precursor to a probable epizooty. Notwithstanding the absence of AIV very virulent isolate detections in wild bird populations the middle term prognosis for 2020 can be designated as cautious, as the avian influenza epidemic situation is deteriorating globally, especially in the European countries, and the threat of the virus introduction to the Russian territory with migratory birds still exists.

Key words: avian influenza, wild migratory bird, lake, epizooty, invasion, parasitic disease.

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Результаты научной экспедиции в природные биотопы Республики Тыва в 2019 году для проведения мониторинга инфекционных болезней в популяциях диких птиц

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РЕЗЮМЕ

Представлены результаты научной экспедиции на озёра Тере-Холь и Убсу-Нур Республики Тыва с целью активного мониторинга особо опасных болезней среди диких перелетных водоплавающих птиц и проведения эпизоотологического исследования акватории выбранных биотопов. Озеро Убсу-Нур является своеобразным индикатором заноса вируса гриппа птиц на территорию Российской Федерации, так как оно служит местом стоянок и гнездований многих диких перелетных птиц в период массовых миграций из стран Центральной и Юго-Восточной Азии. При проведении активного мониторинга проводилось полное вскрытие трупов птиц с описанием органов и систем и отбором проб для лабораторной диагностики. В качестве биологического и патологического материала отбирали пробы помета (пуловые пробы), кусочки внутренних органов от мертвых и отстрелянных птиц, кровь (при возможности). При отборе образцов для исследований проводили идентификацию вида с использованием орнитологического определителя. При патологоанатомическом исследовании мертвых или убитых с диагностической целью водоплавающих птиц выявлена высокая степень глистной инвазии нематодами и цестодами. В двух пробах от чаек серебристых был выявлен геном вируса гриппа птиц типа А и в одной из них идентифицирован подтип H13N6. В одной пробе от чаек выявлен парамиксовирус 1-го серотипа (APMV-1) – возбудитель ньюкаслской болезни. Озёра Республики Тыва являются важнейшими точками пробоотбора биоматериала от диких птиц, поскольку первичное обнаружение вируса высокопатогенного гриппа на данных территориях является серьезным сигналом о возможности дальнейшего распространения возбудителя и предвестником возможной эпизоотии. Несмотря на отсутствие случаев обнаружения высоковирулентных изолятов вируса гриппа в дикой популяции, среднесрочный прогноз на 2020 г. можно охарактеризовать как «осторожный», поскольку наблюдается ухудшение эпизоотической ситуации по гриппу в мире, особенно в европейских странах, и сохраняется угроза заноса вируса на территорию России с перелетными птицами.

Ключевые слова: грипп птиц, дикая перелетная птица, озеро, эпизоотия, инвазия, паразитоз.

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INTRODUCTION

Natural ecosystems, where anthropogenic factor does not influence biotopes, are extremely interesting for veterinary, medical and biological sciences, as they provide the possibility to study patterns of infectious and epizootic process development and peculiarities of infectious agent ecology in its habitat, where natural disease foci can occur and persist¹. Ecosystems of the Republic of Tyva, in particular the Tere Khol and Uvs Nuur Lakes, were deliberately selected for studies of the avian influenza virus ecology. Researchers of the FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH") undertake scientific expeditions to the lakes of the Republic of Tyva every year; the Uvs Nuur Lake is some kind of an indicator for avian influenza introduction to the Russian Federation, because this is the resting and nesting areas for many migratory wild birds during the period of mass migrations from Central and South-East Asian countries. For example in 2006 mass deaths of wild migratory birds (great crested grebe/*Podiceps cristatus* was the prevailing species) were reported in the lake area due to infection with H5N1 highly pathogenic influenza. In June 2009 and 2010 dead birds of various species were found at the lake (great-crested grebe/*Podiceps cristatus*, bean goose/*Anser fabalis*, Eurasian spoonbill/*Platalea leucorodia*, Pallas's gull/*Larus ichthyaetus*, gadwall/*Anas strepera*, great cormorant/*Phalacrocorax carbo*, Eurasian coot/*Fulica atra*, great egret/*Egretta alba*, common pochard/*Aythya ferina*), died due to the infection with H5N1.

¹ Lvov D. K., Ilyichev V. D. Migrations of birds and transmission of infection agents. Ecological and geographical links between birds and infection agents [Миграции птиц и перенос возбудителей инфекции. Эколого-географические связи птиц с возбудителями инфекции]. М.: Наука; 1979. 270 p.

H5N1 virus was isolated from dead great-crested grebes also in 2015. Since 2011 diagnostically relevant titres of specific antibodies to H5, H7 and H9 virus subtypes have been detected in sera from shot birds, which suggests avian influenza virus circulation in migratory waterfowl. The influenza A/H5N8 virus was isolated from dead and shot birds at the Uvs Nuur Lake in May 2016; this subtype is responsible for the epizooty in agricultural poultry in Russia (late 2016 – January, 2019). That is why annual comprehensive monitoring in waterfowl populations is an actual and important task of practical veterinary medicine.

MATERIALS AND METHODS

2019 expedition to the Republic of Tyva was arranged within FAO/IAEA research program (Contract No. 22555/R0). In order to prevent shooting of the Red Book and remnant species for the purposes of active monitoring, the official game manager was included into the expedition team. Complete autopsy of bird carcasses followed by description of organs and systems and sample collection for laboratory diagnostics was performed. Droppings (pooled samples), parts of internal organs from dead and shot birds, blood (if possible) served as biological and pathological material for testing. While sampling, species were identified using an ornithological guide [1, 2]. Samples were transported to the laboratory in sealed water-proof insulated containers with cool packs in compliance with current standards [3]. For the purposes of the epizootological study of the lake areas, conventional epizootological techniques and photo- and video equipment were used.

To prepare suspension, samples of internal organ parts and droppings were homogenized and pH 7.4 sterile saline solution was added. For further testing 1 ml of the



Fig. 1. Tere Khol Lake area, 2019

Рис. 1. Акватория оз. Тере-Холь, 2019 г.

prepared suspension was transferred into a tube and centrifuged at low rpm to clarify.

Total RNA was extracted using RIBO-sorb test kit (Cat. No. K2-1-Et-100) in accordance with the manufacturer's guidelines.

Real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) was performed in one run using OneStep RT-PCR Kit (Qiagen, cat. No. 210212), 25 mM magnesium chloride solution (Promega, in kit with Cat. No. M8296) and primer kits specific for genes M, H, N. 25 µl mixture was made from 7.5 µl of de-ionized water; 5 µl 5× RT-PCR buffer; 2.75 µl of 25 mM of mM magnesium chloride solution, 1 µl of 10 mM dNTP solution, 1 µl of each direct and reverse primer solutions (10 picomol/µl); 0.75 µl of fluorescent probe solution (10 picomol/µl); 1 µl of reverse transcriptase and polymerase enzyme solution; 5 µl of total RNA solution. Reverse transcription was performed for 30 min at 50 °C. For amplification the following temperature and time parameters were used: 95 °C – 10 min (polymerase activation), then 40 cycles, each consisting of 3 stages (95 °C – 10 s, 55 °C – 35 s, 72 °C – 10 s).

RESULTS AND DISCUSSION

The fresh water Tere Khol Lake is located 1,300 m above sea level in the south-east of the Republic of Tyva in the Tere Khol tectonic hollow, not far from the Mongolian border. Lack of anthropogenic factor and feed abundance in the lake area attract wild waterfowl, notwithstanding the poor offshore vegetation. The lake is extremely marshy and water has a specific swamp smell (Fig. 1). During examination no wild bird carcasses were found.

The Russian part of the Uvs Nuur Lake is located in the southern part of the Republic of Tyva at the border with Mongolia (Fig. 2). The lake is situated in the tectonic hollow – Great Lakes Depression, in an endorheic basin, is highly saline and its bitterly-salty water is unfit for human consumption. Every year the lake area diminishes due to drying up. The shores in the Russian part (10 km long) are marshy and hard to access. The climate in the basin is sharply continental with great fluctuations in daily and annual temperatures. While examining the lake shore three dead European herring gulls (*Larus argentatus*) were found.



Fig. 2. Uvs Nuur Lake area (Mongolian border), 2019

Рис. 2. Акватория оз. Убсу-Нур (граница с Монголией), 2019 г.

Table
Number of samples taken in natural biotopes

Таблица
Количество отобранных проб в природных биотопах

Species	Tere Khol Lake	Uvs Nuur Lake
Order Charadriiformes		
Black-headed gull (<i>Larus ridibundus</i>)	3	–
European herring gull (<i>Larus argentatus</i>)	–	8
Common tern (<i>Sterna hirundo</i>)	2	–
Wader (<i>Limicolae</i>)	1	–
Common snipe (<i>Gallinago gallinago</i>)	1	–
Order Anseriformes		
Garganey (<i>Anas querquedula</i>)	2	–
Common pochard (<i>Aythya ferina</i>)	1	1
Order Gruiformes		
Eurasian coot (<i>Fulica atra</i>)	3	–
Order Podicipediformes		
Great-crested grebe (<i>Podiceps cristatus</i>)	1	–
Order Suliformes		
Cormorant (<i>Phalacrocorax</i>)	–	2
Droppings (pooled samples)	28	19
Aquatic organisms	18	–

The following bird species were met during the examination of the lakes' areas: cranes (*Grus*), waders (*Limicolae*), gulls (*Laridae*), mallard (*Anas platyrhynchos*), common pochard (*Aythya ferina*), garganey (*Spatula querquedula*), Eurasian coot (*Fulica atra*), great-crested grebe (*Podiceps cristatus*), ruddy shelduck (*Tadorna ferruginea*), perching birds (*Passeriformes*).

Within the active monitoring in 2019 90 samples of biological materials were taken from wild birds: parts of intestines, kidneys, spleen, sometimes brain and droppings (see Table).

The autopsy of dead and killed for diagnostic purposes birds showed single and combined invasions (cestodes and nematodes) in 50% cases. When adult cestodes were detected in small intestines (Fig. 3), distention and vascular congestion of intestinal loops, catarrh of mucosal membrane, which was congested and edemic (Fig. 4), gallbladder enlargement with discharge of bile into the stomach lumen and pancreatitis (pancreas distention, hyperemia, edema and softness) were observed. White cestodes were 12–20 cm long, the width of their proglottids were bigger than their length (Fig. 5). Heavy 0.5–5 cm nematode burden was revealed in cormorants, gulls and ducks (Fig. 6–8). Moreover, the proventriculus mucosa was hyperemic and extremely edemic with petechial hemor-



Fig. 3. Cestodes in intestines (drepanidotaeniosis)

Рис. 3. Цестоды в кишечнике (дрепанидотениоз)

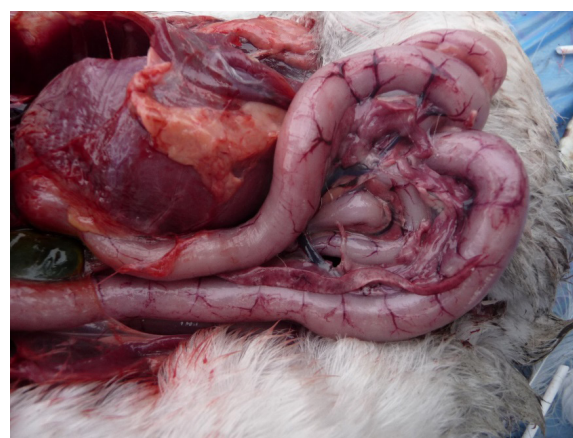


Fig. 4. Distention and vascular congestion of intestinal loops

Рис. 4. Вздутие и полнокровие сосудов петель кишечника

rhages. The gizzard cuticle was boggy and easily detachable; hyperemia and petechia were observed under the cuticle. The tapeworm infestation was assessed as heavy. Such a heavy tapeworm burden in wild bird populations can be explained by the fact that optimal conditions for the propagation of intermediate hosts for avian parasites, in particular of crustaceans, like cyclops, daphnia, gammarids as well as mollusks and other aquatic invertebrates, exist in the concerned ecosystems of the Republic of Tyva. Intermediate hosts for cestodes are cyclops, in organisms of which larvae (cysticercoids) develop; gammarids are intermediate hosts for many tapeworms, responsible for tetrameriosis, streptacarusis and other diseases in birds².

During the necropsy of dead gulls, found on the Uvs Nuur Lake shore, diffuse hemorrhages were observed under the head skin and in the oviduct (Fig. 9–10), inflammation of pancreas and small intestines. Avian influenza type A genome was detected in two samples. Sub-type H13N6 was identified in one of them. Sample taken from the third gull revealed the presence of paramyxovirus serotype 1 (APMV-1), causing Newcastle disease.

² Kotelnikov G. A. Tapeworm tests of animals and environment [Gel'mintologicheskie issledovaniya zhivotnykh i okruzhayushchej sredy]: guiding book. M: Kolos; 1983. 208 p.



Fig. 5. Cestodes in abdominal cavity (European herring gull)

Рис. 5. Цестоды в брюшной полости (чайка серебристая)



Fig. 6. Nematodes in proventriculus (European herring gull)

Рис. 6. Нематоды в железистом желудке птиц (чайка серебристая)



Fig. 7. Nematodes in gizzard (cormorant)

Рис. 7. Нематоды в мышечном желудке птиц (баклан)



Fig. 8. Nematodes in abdominal cavity (great-crested grebe)

Рис. 8. Нематоды в брюшной полости птиц (чомга)

Notwithstanding the PCR positive results, we failed to isolate these pathogens using chicken embryos and cell cultures.

No avian influenza type A genetic material was found in the samples of aquatic organisms (gammarids), taken from the shores. However, there are reports of avian influenza virus accumulation in aquatic organisms, in particular in

mussels and daphnia, inhabiting natural lakes and rivers. For example, A. Pathak et al. studied the survival rate of low pathogenic (H9N2) avian influenza virus in bamboo shrimps (*Atyopsis moluccensis*) and found that this species is able to accumulate the influenza agent and support its circulation in the biotope [4].

Notwithstanding the fact that no highly avian influenza virus virulent isolates were detected in wild fauna, the middle term forecast for 2020 can be characterized as "cautious", because the risk of avian influenza virus introduction into the Russian territory together with migratory birds still remains. The further spread of H5Nx avian influenza viruses, potential epizootic emergence and severity of the epizootic process will depend on direct and indirect contacts between wild virus-carrier birds and poultry.

CONCLUSIONS

1. Heavy cestode and nematode burden of wild migratory bird populations in the Republic of Tyva was established.

2. Major post-mortem lesions due to inflammations in organs and tissues are associated with parasitic diseases in studied birds.

3. Detection of avian influenza virus subtype H13N6 and Newcastle disease agent in gulls suggest circulation



Fig. 9. Hemorrhages under head skin

Рис. 9. Кровоизлияния под кожей головы



Fig. 10. Hemorrhages in oviduct

Рис. 10. Кровоизлияния в яйцеводе

of these viruses in the wild bird populations and this virus carriage state is a common case.

4. Lack of mass deaths in wild birds and negative results of laboratory tests for highly pathogenic avian influenza enables to predict a low risk of the agent introduction from the Central and South-East Asian countries through the migration routes, located across the Republic of Tyva in 2017–2019 [5–7].

5. The middle term prognosis for 2020 can be characterized as “cautious”, because the avian influenza epidemic situation is deteriorating globally, especially in the European countries, and the threat of the virus introduction to the Russian territory with migratory birds still exists.

6. The lakes of the Republic of Tyva are the most important sites for sampling of biological material from wild birds, because the primary detection of highly pathogenic avian influenza virus in this territory is an essential signal of the agent further spread and a precursor of a potential epizooty.

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