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ВЕСТНИК

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VIRUSES OF MARINE MAMMALS AND METAGENOMIC MONITORING OF INFECTIOUS DISEASES

Abstracts. The review presents information on epizootic diseases in marine mammals caused by influenza viruses of genera A and B, morbilliviruses, adeno-, herpes-, calici- and coronoviruses. Virological and serological data about the spectrum of viral infections in marine mammals are given. Describes epizootic events in marine mammals induced by influenza A viruses and isolation of strains with antigenic formulas H1N1, H1N3, H3N3, H3N8, H4N5, H4N6, H7N7, H10N7, H13N2, H13N9. It is suggested that the seals may be infected with a wide range of influenza viruses by direct transmission without prior adaptation. It is emphasized that marine pinnipeds can serve as one of the reservoirs of human influenza B virus in nature. Data on the classification of pinniped and cetacean coronaviruses are presented. A variety of pinnipeds herpesviruses and the clinical significance of their morbidity indicators in the diagnosis of viral infections of seals are shown. It emphasizes the need for the development of proactive diagnostic tools for the timely detection of new viruses with zoonotic potential that can cause disease outbreaks. The negative effects of diseases with unknown etiologies on the population of Caspian seals are noted. The conclusion is made about the need for metagenomic monitoring of Caspian seal virome in order to establish the role of viral pathogens in their morbidity and regular mortality under conditions of intensive habitat development.

Key words: virus**,** marine mammals, Caspian seal, virom, metagenomic monitoring.

Health survey of marine mammal populations is one of the most important and significant scientific directions in ecology. Current knowledge about the diversity of pathogens of marine mammals is expanding rapidly [1, 2]. Viral diseases play a major role in regulating the dynamics of the population of wild animals, limiting their growth and enhancing selection at the genetic level. The impact of viruses becomes even more sever to the species endangered or fragmented at the result of anthropogenic pressure.

Viruses from marine mammals were isolated for the first time in 1972 from Californian sea lion *(Zalophuscalifornianus)*, on San Miguel Island during an outbreak of infection among these animals. The etiological agent attributed to caliciviruses and adopted as the San Miguel type I sea lion virus. They were isolated from 11 species of pinnipeds and cetaceans [3].

Paramyxovirus infection caused by members of the genus morbillivirus is widespread among marine mammals and causes serious threat to the population of these animals. To date, four species of morbilliviruses that infect marine mammals have been described: canine distemper virus in the Baikal *(Pusasibirica)* and Caspian seals *(Pusacaspica)*; cetacean morbillivirus - in dolphins and whales; phocine distemper virus - in harbor seals *(Phocavitulina)*; porpoise morbillivirus- in porpoises *(Phocoenaphocoena)*.

Morbilliviruses in marine mammals were first discovered in the late 1980s during the mass mortality of harbor seals and gray seals (*Halichoerusgrypus)* on the North European coasts in 1987-1988.

Phocine distemper virus (PDV) caused widespread epizootics among harbor seals in the coastal waters of Northern Europe in 1988, which killed more than 12 thousand animals [4]. In 2002, this virus returned to the indicated population, causing the death of about 1000 animals on the coasts of Scandinavia [5].

Morbillivirus epizootics (including canine distemper virus) occurred in populations of pinnipeds and cetaceans of the Northern Hemisphere [6]. The canine distemper virus caused the mass death of the Baikal seal (*Pusasibirica*) in 1987-1988 in Siberia, this virus was also involved in the mortality of crabeater seals

(*Lobodoncarcinophagus*) in 1955 in Antarctica. It was thought that the virus was transmitted to the population of pinnipeds through contact with domestic dogs [7].

Morbillivirus infections with high mortality have been registered among porpoises, stripeddolphins *(Stenella coeruleoalba)* and bottlenose dolphins (*Tursiopstruncatus*) [8, 9], harbor seals and harp seals (*Pagophilusgroenlandicus*) [10]. The mass die-off of Caspian seals in the spring and summer of 2000 was caused by canine distemper virus[11].

An infection potential of influenza A virus for the seals was made known from the epizootic outbreak on the coast of New England in 1979-1980resulted death of 600 animals [12]. The genetic characterization of the virus showed that all its RNA segments showed a close resemblance to those of avian strains. The second epizooty among the seals, occurring with pronounced clinical manifestations of pneumonia, occurred on the coast of New England in June 1982-August 1983 was associated with the influenza A virus (H4N5) [13].

Five strains of influenza A virus were isolated from seals that died from pneumonia on the Cape Cod Peninsula (Massachusetts, USA) from January 1991 - February 1992, two of which were identified as A (H4N6), and three as A H3N3. The HA genes of influenza A(H3N3) viruses were 99.7% identical to each other. Phylogenetic analysis demonstrated a close relationship between their sequences and those of the hemagglutinin H3 avian isolate. These data indicate that the influenza virus subtype H3 has been circulating for a long time in seal populations [14].

Identification of various types of influenza viruses in the seal populations indicates the possibility of their participation in genetic reassortment. Molecular- genetic studies established an avian origin of seal influenza isolates [13].

Pandemic H1N1 influenza A virus isolated from northern elephant seals *(Miroungaangustirostris)*, near the California coast of the United States in 2010. Full genome sequencing revealed more than 99% homology with pandemic “swine” influenza virus A/California/04/2009which circulated among people since 2009 [15].

In the fall of 2011 in New England (Massachusetts, USA), 162 seals died from pneumonia caused by the influenza A virus (H3N8). The pathogen was found to be similar to influenza viruses of waterfowl that circulated in North America since 2002 and had a mutation in the PB2 gene, characteristic of the highly pathogenic for humans variant H5N1, which indicated its potential for interspecies transmission and adapting to mammals [16].

Until recently, influenza A viruses did not isolate from the pinnipeds of the Palearctic. Previously, virological evidence of their involvement in outbreaks of infection among seals was obtained only in the North American continent.

Epizootics of marine mammals caused by influenza A viruses in other parts of the world, were first reported in 2014. The influenza A virus (H10N7) is isolated from found dead harbor seals (Phocavitulina) on the North Sea coast in Sweden, Denmark, Germany [17-19] where over 1400 animals died.

Influenza A (H1N3) virus is isolated D.K. Lvov et al. [20] from a dwarf whale *(Balaenopteraacutorostrata)* in the South Pacific in 1975-1976. From toothed whales (long-finned pilot whale - *Globicephalamelas*) V. Hinshaw et al. [21] identified the H13N9 and H13N2 influenza viruses genetically close to the H13 viruses circulating among gulls.

The data about isolation of influenza A virus (H7N7) from the materials collected during mass mortality of Caspian seals in April-June 2000-2002 was reported by Shestopalov A.M. et al. [22, 23] and Chuvakova Z.K. et al. [24, 25] in conference papers and review article. Despite of an extreme importance of those findings any other data about further characterization of genetic or pathobiological peculiarities of epizootic strain have not been published.

Later, influenza A (H4N6) viruses were isolated from seals in the Russian waters of the Caspian Sea in 2002 and 2012 [26, 27]. Phylogenetic analysis demonstrated that all genes ofseal origin A(H4N6) were closely related to avian-derived influenza viruses of the classical Eurasian lineage circulating in wild birds.

In contrast to influenza A viruses, which have been isolated from many different species, influenza B viruses were human pathogens with no or unknown reservoirs in nature until 1999. However, further it was argued that common and gray seals can become infected with this virus [28]. Influenza virus B was isolated from juvenile harbor seal with symptoms of respiratory disease and *in vitro* infected cell culture of

phocine kidney cells. Since the moment of acknowledgment of marine mammals as new hosts, there have been several reports on the detection of antibodies to the influenza B virus in some species of *Otariid* and *Phocid* species [29, 30]. Based on these data, it was suggested that the seals may serve as a reservoir of the human influenza B virus.

Adenoviral infections with clinical signs of hepatitis and enteritis have been noted in sea lions [31]. Outbreaks of this infection have also been observed in fur seals, sei whales(*Balaenoptera borealis*), bowhead whales*(Balaena mysticetus)*, beluga whales(*Delphinapterus leucas*), bottlenose dolphins [32-34].

Herpesviruses are isolated from seals with clinical signs of acute pneumonia and hepatitis in the Netherlands in 1985. [35]. They are subsequently isolated from bottlenose dolphins, killer whales*(Orcinus orca)*, California sea lions and gray seals [36]. At present, seven species of herpes viruses (PhHV-1 - PhHV-7), belonging to the genera alpha and gamma herpes viruses, have been isolated from phocids [35-40]. In the infectious pathology of these animals, herpesvirus seals 1 [Phocid Herpesvirus type 1] (PhHV-1) of the genus α-herpesviruses are most significant pathogen. Infection caused by these viruses in the harbor seals and gray seals is characterized by respiratory manifestations with interstitial pneumonia and coagulative necrosis of the adrenal tissues and liver [35, 41].

The rest six herpesviruses (PhHV-2 - PhHV-7) belong to the genus γ- herpes viruses, of which PhHV-4 and PhHV-7 have diagnostic importance in inflammation and ulceration of oral cavity soft tissues of seals. PhHV-6 herpesvirus in seals is associated with eye disorders [39]. The role of the PhHV-2, PhHV-3 and PhHV-5 viruses in the infectious pathology of seals remains unknown.

Herpesvirus PhHV-1 was isolated from newborn harbor seals with clinical signs of acute pneumonia and hepatitis at a rehabilitation center in the Netherlands in 1985 [35]. In the Pacific harbor seal *(Phoca-vitulinarichardsii)*; this virus is associated with focal caogulatory necrosis of the adrenal cortex and liver. In phylogenetic studies of PhHV-1DNA polymerase (gB) gene, their close relationship with herpesviruses of dogs and cats has been established.

Serological studies have shown widespread infection of PhHV-1 or other closely related α-herpes viruses in the free-ranging seal populations of the North Sea, as well as in the waters of the Antarctic and the North Pacific [42-44].

The results of virological screening, molecular genetics and serological studies indicate the circulation of PhHV-1 among Caspian seals and its possible involvement in their infectious pathology and recurrent mortality [45].

Coronaviruses are common pathogens of the respiratory system, gastrointestinal tract and cause systemic infections of domestic animals and wildlife.

Coronaviruses in marine animals were first described during seal mortality investigation in an aquarium of Florida [46], as well as among free-ranging pinnipeds along the coast of central California []. In harbor seals (harbor seal coronavirus - HSCoV), the infection is manifested by hemorrhagic pneumonia and causes epizootics with high mortality. In cetaceans, coronoviruses were isolated from the beluga whales in captivity [47] and from the feces of three Indo-Pacific dolphins (*Tursiopsaduncus*) [48].

HSCoV belongs to the genus of α-coronaviruses, closely related with coronoviruses of cats, dogs, ferrets and pigs [1]. Beluga whale coronavirus and dolphin coronavirus are γ-coronaviruses and represent a special type of cetacean coronavirus [50].

In addition to the listed viruses that infect marine mammals, there are reports of astro-and poxvirus zoonoses of cetaceans and pinnipeds [49-51].

Taken together, the available evidence suggests that the causative agents of these diseases are detected only after mass outbreaks of infections that have reached an epizootic scales. Therefore, it requires proactive diagnostic tools for the timely detection of new viruses with zoonotic potential capable of causing outbreaks of diseases, in order to develop an effective strategy to combat the devastating epizootics of vulnerable animal species.

Routinely, for the diagnosis of viral diseases, cell cultures are used to isolate pathogens, specific antibody based immunological tests, and polymerase chain reaction (PCR) for amplifying viral gene segments [52]. Unfortunately, all of them turned out to be ineffective in identifying previously unknown viral pathogens due to their unculture ability and lack of diagnostic antibodies to them. The possibilities of PCR diagnostics in such cases are also limited due to the need for specific oligonucleotide primers complementary to the conserved sequence of the pathogen genome fragment.

Unlike the above methods of identifying pathogens, viral metagenomics allows to identify new viruses without prior knowledge of their genome sequences. Viral metagenomics is used to directly identify new etiological agents from the affected animal tissues with pathological alterations [53, 54]. For example, in metagenomic studies of Ng et al. identified annelovirus (ZcAV) infection in two dead sea lions [55].The authors showed that ZcAV is found only in the lungs and pleural cavity, absent in the tonsils, lymph nodes, liver and other organs. This indicates that metagenomics can be used to determine the tissue tropism of new viruses. Subsequent studies have shown that the prevalence of the virus was high during outbreaks among sea lions in captivity (100%), low in wild representatives (11%); all this indicates the possibility of using metagenomic studies to detect viruses in marine animals. In addition to ZcAV, seal anneloviruses and fur seal sacobuviruses have been discovered using metagenomic studies [56]. Bodewes et al. [40] using metagenomic studies identified herpesvirus seal type 7 as an etiologic agent for ulcerative gingivitis in seals. With a similar method of analysis, it is also shown that Enhydralutis papilloma virus 1 is the etiological agent of oral cavity tumors in sea otters.

In the context of the above provided, the Caspian seal, as an endemic marine mammal, is one of the indicators of the Caspian Sea ecosystem. Diseases of unknown etiology [57-59] have a negative effect on its population, which indicates the importance of metagenomic studies of Caspian seal virom seals to establish the role of viral pathogens in their morbidity and regular mortality in conditions of intensive habitat development.

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ТЕҢІЗ СҮТҚОРЕКТІЛЕРІНІҢ ВИРУСТЫҚ ИНФЕКЦИЯЛАРЫ ЖӘНЕ МЕТАГЕНОМИКАЛЫҚ МОНИТОРИНГТЕ ЖАҢА ВИРУСТАРДЫ ТАБУ

Аннотация. Шолуда теңіз сүтқоректілеріндегі тұмау А және В вирустары, морбилливирустар, адено-, герпес-, калици- және короновирустар қоздырған індеттер туралы мағлұмат берілген. Теңіз сүтқоректілерінің вирустық инфекцияларының ауқымы жайында вирусологиялық және серологиялық деректер келтірілген. Теңіз сүтқоректілердегі тұмау А вирустарының індетімен олардан антигендік формулалары H1N1, H1N3, H3N3, H3N8, H4N5, H4N6, H7N7, H10N7, H13N2, H13N9 штамдарды оқшаулап алу жағдайлары сипатталады. Итбалықтардың алдын-ала бейімделмеген тұмау вирусының ауқымды спектрін тікелей жұқтыру мүмкіншілігі топшыланған. Теңіз ескекаяқтылары адамның В тұмауы вирусының табиғаттағы сақтаушысыдеген түсініке басымдық берілген. Ескекаяқтылар мен киттәрізділер коронавирустарының классификациясы туралы мәліметтер ұсынылған. Итбалықтар герпесвирустарының түр алуандығы мен олар туғызатын ауруларды балауда клиникалық белгілерінің маңызы көрсетілген. Ауру тудыруы мүмкін зооноздық әлеуеті бар жаңа вирустарды дер кезінде анықтау үшін ұтқыр диагностикалық құралдарды әзірлеу қажеттілігіне баса назар аударады. Каспий итбалықтары популяциясына этиологиясы белгісізаурулардың теріс әсерлері жөнінде айтылған. Шолуға тіршілік ортасын қарқынды игеру жағдайындағы каспий итбалығы ауруларымен тұрақты өлім-жітіміне вирустардыңқатысынзерделеу үшінолардың виромына метагеномикалық мониторинг жүргізу қажет деген қорытынды жасалынған.

Түйін сөздер: вирус, теңіз сүтқоректілері, каспий итбалығы, вирус, метагеномдық мониоринг.

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ВИРУСНЫЕ ИНФЕКЦИИ МОРСКИХ МЛЕКОПИТАЮЩИХ И ОБНАРУЖЕНИЕ НОВЫХ ВИРУСОВ ПРИ МЕТАГЕНОМНОМ МОНИТОРИНГЕ

Аннотация. В обзоре представлены сведения об эпизоотиях среди морских млекопитающих, вызванных вирусами гриппа родов А и В, морбилливирусами, адено-, герпес-, калици-, короновирусами. Приводятся вирусологические и серологические данные о спектре вирусных инфекций морских млекопитающих. Описываются случаи эпизоотиию морских млекопитающих вирусами гриппа А и изоляция от них штаммов с

антигенными формулами H1N1, H1N3, H3N3, H3N8, H4N5, H4N6, H7N7, H10N7, H13N2, H13N9. Высказывается предположение о возможности инфицирования тюленей широким спектром вирусов гриппа путем прямой трансмиссии без предварительной адаптации. Подчеркивается положение о том, что морские ластоногие могут служить одним из резервуаров вируса гриппа В человека в природе. Приведены данные о классификации коронавирусов ластоногих и китообразных. Показано разнообразие герпесвирусов ластоногих и клиническая значимость показателей их заболеваемости в диагностике вирусных инфекций тюленей. Подчеркивается потребность в разработках упреждающих диагностических инструментов для своевременного выявления новых вирусов с зоонозным потенциалом, способных вызывать вспышки болезней. Отмечаются отрицательные воздействия заболеваний с невыясненными этиологиями на популяцию каспийских тюленей. Делается заключение о необходимости проведения метагеномного мониторинга вирома каспийских тюленей для установления роли вирусных патогенов в их заболеваемости и регулярной смертности в условиях интенсивного освоения среды обитания.

Ключевые слова: вирус, морские млекопитающие, каспийский тюлень, вирома, метагеномный мониторинг.

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