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Emerging-reemerging Viral Infections in Northern Eurasia: Global Consequences.

The problem of emerging and re-emerging infections has today a high priority. It is considered that they are unpredictable and are able to provoke extraordinary epidemic situations and the struggle against such situations at the stage of their appearance is difficult or impossible. Prevention of bioterrorism consequences is important block of this problem. But most dangerous terrorist is nature. For prognosis and analysis of extraordinary epidemic situations is necessary permanent monitoring of agents ecology with using molecular-genetic methods for determination of genetic properties of virus populations changing in the time and the space, and development on this base of adequate diagnostic kits and vaccines. We started such work 40 years ago. The theoretical basis of carrying out the monitoring in different ecosystems with the use of molecular ecology and epidemiology methods was carried out. Methodological approach provided ecological sounding of the territories with the collection of field materials in meridional sections of about 2 000 km length in the whole territory of Northern Eurasia. In this way the territory of more than 15 million km² was investigated. Before our work this territory was “big white spot” concerning arboviruses and some other pathogens.

Twelve zonds were performed which passed through landscape belt of arctic, tundra, taiga, leaf-bearing forest, steppe, desert within 18 physico-geographical lands with unique ecosystems. About 70 viruses were isolated from which 24 viruses proved to be new for science. Etiological role of isolated viruses in human pathology was revealed. 6 unknown before infections were described. Potential danger of emerging situations in various ecological landscapes belts was determined for different ecological complexes of arboviruses, transmitted by mosquitoes and ticks. Forecast of the area of some new viruses has been predicted. Transmitted by mosquitoes Karelian fever virus (Togaviridae, Alfavirus, Western encephalomyelitis complex) which very close to African Babanki virus, introduced by birds to Fennoscandia from Africa through East European migratory root. Issyk-Kul fever (Bunyaviridae) from Central Asia transmitted by obligate parasites of bats ticks *Argas vespertilionis* complex, isolated also in Malaysia, Afghanistan and South Africa. Mosquito-transmitted viruses from California complex (Bunyaviridae, Bunyavirus) was shown practically everywhere in belts of tundra, taiga and leaf-bearing forest. Most pathogenic Snow-shoe hare virus distribute to east from Yenisei river – in East Siberia and far north Pacific area of Russia. The infection rate of mosquitoes and the level of infection rate of population are the highest in taiga and tundra belts, where up to 80% of the population is infected during their life. According to our calculations tens thousands of people every year became ill with this infection. On the north-eastern Siberia we determined active circulation of Getah Togavirus antigenically related to Chikungunya and Ross-river viruses, associated with hemorrhagic fevers in South-East Asia and Australia. Penetration of virus occurred with migrating birds through East Asian - Pacific migratory roots.

The recent examples of the emerging epidemic situation are appearance of extensive outbreak of West Nile and Crimean-Congo hemorrhagic fevers in the south of Russia. The causes can be the combination of anthropogenic and natural factors. Natural CCHF foci in the world and in our country practically coincide with the area of the main virus host and vector, the ticks *Hyalomma marginatum*. In the south of Russia endemic areal include landscape belts of dry steppe, semi-desert and desert. In the north the area is limited by isotherma of sum of effective temperatures 3000⁰. For the last years a number circumstances led to a considerable increase of ticks' quantity. This and some other factors produced the emergence epidemic situation. But the opportunity of the change of genetic features of virus population also exists. We tested it in comparative studies of the genome of epidemic strains isolated for the last 4 years from patients and ticks and strains isolated earlier in the south of Russia and Central Asia and also compare with date on strains from the South Asia and Africa. It was found that in the south of Russia, as it was 30 years before, genetically similar strains are circulating. Central-Asian strains similar to Chinese strains and different from Russian, South-Asian and African strains. Thus, the evolution of CCHF virus is going very slowly.

West Nile fever. Endemic territories in the south of Russia are known from the moment of first isolation of this virus in north of Caspian sea basin region 40 years ago. Sporadic morbidity and small outbreaks were observed in southern regions of the Soviet Union practically every year. Immunologic structure of the population of the USSR was known, highest indices found in the south of Russia, particularly in the Volga delta region.

Therefore the epidemic of 1999 originally diagnosed by local specialists as enterovirus infection did not prove to be unexpected for us. But the epidemic had essential features: mortality was unusually high – about 10%. Only laboratory verified cases were over 500 in this year. According to our calculations on results of serological examination before and after outbreak, real morbidity could be in three-ten times more and number of infected persons assumed about 200 thousand. A mysteriously simultaneous similar epidemic outbreak occurred in New York. Homeland of this virus is Africa. The virus was not brought to the American continent by natural way for the last 80 million years from the time of Pangeya and Honvane division, in Chalk period of Mezozoic era, when America floated away from Africa through Atlantic ocean. On next year in the USA secondary focus appeared in the Florida after introduction of virus by migrated birds along Atlantic fly-way and than in next spring outspread for all USA along Atlantic, Mississippi, Central and Pacific migratory roots. After 2-3 years all continent became for a long time in endemic territory. And to the south of Europe, first of all to delta of large rivers like Rhone, Danube, Volga and others, constant introduction of viral population through main migratory roots of birds takes place with the subsequent including in the virus circulation of local populations of birds and mosquitoes. Phylogenetic analysis of Russian strains showed, that recent epidemic strains similar to epidemic strains from New York, Israel and Romania and different from the strain, isolated in the same region from various natural sources 20-30 years ago in the period of absence of epidemic situation, which probably is stipulated not only by natural-social factors, but also by the change of genetic features of viral population.

But now will be another story - about avian flu.

Influenza A viruses have the richest gene pool in natural biocenoses. Some of them could be dangerous for domestic animals, and potentially for humans. Moreover, the threat of some natural or laboratory-modified variants being used for bioterrorism purposes with terrible consequences cannot be ruled out. Among wild birds usually circulating low pathogenic avian influenza strains (LPAI). But after the penetration in high susceptible populations of poultry this strains turn to high pathogenic avian influenza (HPAI). All of them could exchange of genetic material in process of reassortation, so all flu A viruses have common

protected gene fund. Some of known viruses of birds could be progenitors for emerging and reemerging epizootic and pandemic viruses.

It is why 35 years ago we started the monitoring of virus circulation in key points of main migration routes of birds in Northern Eurasia. Complex investigation included: virus isolation and identification, study of genomic structure, receptor activity, pathogenicity, serological investigations of wild and domestic animals and humans, detection of virus RNA, action of antivirals.

In different regions of Northern Eurasia for last 35 years we isolated 15 from 16 known Influenza A viruses including H5 from wild ducks in Siberia and Far East.

Molecular-genetic study showed that they belong to low pathogenic strains avian Influenza (LPAI). HA cleavage site was usual for LPAI strains.

Phylogenetic analysis of HA of Siberian strain showed its similarity with avirulent viruses circulated in Malaysia and human virulent Hong Kong strain isolated six years later.

North Asian LPAI population could be precursors for high pathogenic avian strains (HPAI) provoked catastrophic outbreak in South-East Asia. We also predicted high level of risk penetration of HPAI from South-East Asia to Russia, especially to Siberia and Far East by spring migration of birds.

And then was happened that must be happen – epizootic outbreak among poultry year ago on Western Siberia.

Isolation of the virus strains was performed using RT-PCR and bio-chip positive field samples in embryo pig kidney cells and MDCK continuous cell lines. Strains were isolated from sick poultry and clinically healthy grebe *Podiceps cristatus* and deposited into Russian State Collection of Viruses.

Sequencing revealed that hemagglutinin cleavage site of all strains has common sequence with the multibasic amino acid stretch that is usual for HPAI strains.

HA gene nucleotide sequences of all strains from poultry and grebe were practically identical. The extent of genetic identity of the isolated strains justifies proposed link between viruses circulating among wild birds and poultry. However, these strains differ from strains known before and have high proximity with Qinghai strains isolated in spring epizootic of wild birds on Kukuor lake in North Eastern Qinghai province of China. It's a border between Tibet and Dzhungaria.

Complete genome sequence of HPAI Qinghai - Siberian strains from wild birds and poultry confirmed these results. All of eight segments "sitting" in the same "nests" on the same "twigs".

All strains of Siberian - Qinghai clade have several notable features including: 1. a multiple basic amino motif in the cleavage site of the HA which is characteristic of the HPAI lineage; 2. a twenty amino acid deletion in the stalk of the NA segment associated with adaptation to poultry (genotype Z). 3. Glutamin acid -92 in NS1 related to increased resistance to the antiviral action of interferon; 4. Ser-31 in M2 associated with rimantadine / amantadine sensitivity (it's only single good news); 5. Lys-627 in PB2 associated with increased virulence for mammals. Results of molecular-genetic study good correlated with biological properties of Siberian strains. Isolated strain deposited in State Virus Collection used in Russia for large-scale production of veterinarian vaccine, which used for mass vaccination of poultry with good results.

Five cell lines of mammalian origin used for the examination of avian influenza strain replication. Strains replicated well in all five without trypsin. Sensitivity increased in the order hamster kidney BHK-21 → human embryo lung → Green monkey kidney Vero-E6 → canine kidney MDCK → pig embryo kidney. Anti-virus action of antivirals increases in the order of remantadine → amantadine → ribavirin → arbidol → ozeltamivir.

This is the Volga river delta, where mass mortality of Mute swans *Cygnus olor* was observed in last December after short stop there of northern species of Tufted ducks *Aythya fuligula* Swans - two hours prior to death. But they picked by crows. You can see blood.

Strains isolated from swans not differ for all genes from Qinghai – Siberian clide. So, HPAI strains circulated among wild birds on the very wide territory from spring to autumn of the last year not significantly changed and not lost high pathogenicity,

Phylogenetical similarity Qinghai and Siberian strains strongly suggests that the rout of introduction of HPAI population from Qinghai into Northern Eurasia is via long-range spring migration of birds through the so called “Dzhungarian Gate”, between Tien Shan mountains and Takla Makan desert in the West and Mongolian Altai mountains and Gobi desert in the East.

So, how I can imagine development of events

At first the LPAI strains circulated in Siberia and Far East among wild birds were brought during autumn migrations, to the South-East Asia. There they transformed among poultry into circulation in natural biocenoses. Than HPAI strains with wild birds from Qinghai lace in China through “Dzhungarian gate” penetrated splashed into West Siberian lowland and outspread to the nesting places in the territory of Northern Eurasia more than 10 millions km² up to coast of Polar ocean and than in last autumn the birds fly back to the overwintering places and again carry virus, which already flew to most Asian and European countries and Africa. This is Siberian drops of virus. Last epizod we found in June in East Siberia in the Mongolian border (51⁰ N, 93⁰ E), where thousands birds died. Complete genome sequencing showed that isolated strain also belong to Qinghai – Siberian clide. So virus not lost high pathogenicity after year circulation in natural biocenoses.

Every water reservoirs in the places of nesting, overwintering and in the routs of birds' flights could be polluted by the virus. And virus would survive there during the Russian cold winter up to the spring. Every reservoir where there are faces of the infected birds could turns to be the “delayed-action mine”. It may be compared with involving of peat-bogs in to a forest fire. When infected and healthy birds return back through this “mine fields” and the events in future would be much more menacing than in the previous season. When the HPAI strains circulating now among wild birds will become to LPAI again? It is impossible to forecast how much time this process will take, months or years. Study of this and other viruses evolution is the priority object. It's necessary for prognosis of events - panzooty, pandemy in future. The key located in the territory of Northern Eurasia, which is “a big kitchen” and South-East Asia and other country – “a dining-room”.

Different species and their populations from various ecosystems have close contact in places of rest and overwintering habitats. Birds nesting in Europe and West Siberia are mostly overwintering in Southern Europe, Black and Caspian see basins, Crimea, Caucasus, Turkey, Israel (short-distance migrants) and in Africa, South-West, South-East, Central Asia, Indian subcontinent (long-distance migrants). Birds from Eastern Siberia and Far East overwintering mostly on East-Central China, Korea, Japan (short-distance migrants), Pacific islands, partly in North-Central-South America regions, Australia, New-Zealand (long-distance migrants). Populations of birds from different regions have active contacts – overlapping of flyways and overwintering areals. These are excellent conditions for exchanges of adapted viruses and outspreading of panzooty on different countries and all over the world.

Concerning the pandemic virus, it may appear in Russia and others polluted by HPAI virus countries. But more likely this variant will form in China, where the opportunities of transformation of reassortants are especially high, taking into account the activity of epizootic process and huge density of susceptible population. The appearance of pandemic virus may occur at any moment, maybe tomorrow, today or yesterday.

Ecology of viruses studies interactions of virus populations and their hosts, and influence of abiotic (mostly climatic) factors upon these populations. Further development of this field provide as opportunity to understand where are ecological niches of populations, how does change of their properties occur, why does their evolution proceed in either trends. Answers of this questions – where, how, and why – are necessary for the explanation and in due course for prediction of conditions, which lead to the development of epizootic and epidemic situation and minimize their consequences.

Of course, it's need of global cooperation and special support. The time does not wait.