Avian influenza: the analysis of the stages and mechanisms of possible virus transformation into pandemic agent

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It was proved in retrospect, that the previous pandemic viruses type A are in their structure either reassortants of human and avian influenza viruses (H2N2 – 1957, H3N2 – 1948), or avian virus adapted to humans (H1N1 – 1918) [1, 2]. Meanwhile it was established that avian influenza viruses (AIV) have very low infectivity for humans and are not able to spread among people. Extremely rare cases of disease of humans caused by avian influenza usually appear by alimentary path of infection and rarely by air-drop. There is need in additional knowledge about the process of transformation of AIV into the agent of pandemic for well-founded prognosis of pandemic and undertaking early prophylactic actions. Analysis of supposed conditions necessary for the appearance of influenza virus with pandemic potency was carried out previously [3-7]. We examine in this article the basic stages and mechanisms of this event on the base of literature data.

1. Avian influenza. Influenza is a high contagious human, avian and mammal infection [8]. Three serological types of influenza viruses: A, B and C cause the disease. Birds and mammals are infected by influenza virus type A only. Natural reservoir of influenza virus type A consists of 16 subtypes of hemagglutinin (HA) and 9 of neuraminidase (NA). There are hundreds of strains of influenza virus and thousands of its isolates. Among the poultry and wild birds more than 90 representatives of 12 species birds are getting ill with influenza. Turkeys and chickens are the most sensible to influenza virus. In poultry influenza often exists in the form of epizootic accompanied by high mortality. At the same time there are a wide variety of viruses, differing by extent of pathogenicity. Influenza virus doesn't often cause the clinical sign of disease in wild birds. Infected birds can overcome long distance during migration and be a carrier of disease. The ducks are considered the main natural reservoir and carrier of the disease to poultry. Therefore, first influenza diseases often begin in the groups of the freely walking poultry in countryside, located near open waters.

Influenza of the poultry, caused by the highly pathogenic virus, passes quickly and leads to the fatal outcome (100% mortality) on the background of the organism dehydration and intoxication. The visible alterations are poor. The most typical signs of the disease are the abrupt fall of activity, the affection of crests with petechial hemorrhages and edema, resulting in the fast

ulceration and necrosis. Respiratory symptoms (nasal discharge, rales, cough spasms, sneezing) are mostly connected with low pathogenic influenza virus (75% mortality). The disease signs at that varies from strong to weak.

Subtypes H5 and H7 (H5N1, H7N2, H7N3) belong to high pathogenic avian influenza viruses. The data about mass epizootic caused by highly pathogenic AIV are presented in the table 1. During 9 years avian influenza has spread all over the world causing damage to economic. Incubation period of the disease caused by these agents was from several hours to 3 days. It depends on the pathogenicity level of the virus, virus dose, the route of infecting, bird species and status of its resistance. Virus is mainly reproduced in gastrointestinal tract. It's excreted in large amounts with excrements, falling into soil, water and birdseed. The birds are mainly infected by influenza via alimentary route.

2. Pathogenicity of AIV for human. It was considered, that in case of human infection acquired from birds, the evanescent conjunctivitis and the symptoms of slight indisposition arise. Since 1997 the reports have been appeared about cases of the heaviest forms of human avian influenza, often with fatal outcome [1, 2, 9, 10]. Since 2003 to 23.08.2006 (on laboratory confirmed data WHO) 241 persons have been got ill, 141 from them have died. AIV has developed high pathogenicity for human and the ability to affect different systems and organs: intestine, lungs, kidneys, brain et.al. Lethal outcome is observed mostly from acute lung and kidney failure in about 50% of cases. Children are the main victims of avian influenza. Workers who had not had the professional close contact with infected bird, did not usually develop the severe forms of avian influenza. The disease does not circulate among people. No local epidemic episode was detected, caused by transmission of avian influenza from human to human. Currently man is a deadlock in transduction of this infectious agent. Leading experts of different countries, including WHO experts, express concern that avian influenza virus will obtain ability to spread among people and become anthroponotic in the near future. On their opinion, actively expressed since 1999, the world is now on the barrier of total pandemic. WHO determines the situation of influenza by the new classification as "Period of pandemic threat, phase 3" [10]. It should be taken into account that although the cases of human infecting with new virus subtypes are revealed in that period, human-to-human transmission of the virus is not observed or observed very rarely after close contacts.

3. Main conditions of pandemic appearance. Influenza remains the unique infection causing pandemic during last two centuries [1, 2, 11, 12]. Influenza pandemic are characterized by global spread of disease involving all groups of people. Frequency of severe clinical forms of the disease and mortality are essentially enhanced comparing to annual epidemic. Danger of the economical and social shock in the society life appears. The appearance of pathogenic virus type

A with increased capacity for rapid spread among people by respiratory way combined with the absence of population immunity to infectious agent is a cause of pandemic. Human organism infected by the virus, even of zoonotic origin, is a unique source of infection in epidemic and pandemic and we tell about purely anthroponotic influenza infection. Taking into account three parts of epidemic process "the source of infectious agent – the mechanism of its transmission – susceptible population", it can carry to the main conditions of the pandemic appearance:

- appearance in human population of enough pathogenic influenza virus type A, with modified hemagglutinin or hemagglutinin and neuraminidase with respect to the population immune to influenza;

- active transmission of the agent among people by respiratory way with short incubation period and high contagiousness of the acute disease.

As results from the first definition the property of high pathogenicity for humans appeared since 1997 is not mandatory for pandemic appearance. It's important only that the virus was pathogenic enough. As results from the second definition, it must be enough of virus progeny in the secret of respiratory tract of ill people for human-to-human transmission of the agent by respiratory way.

4. Main stages of possible conversion of the avian influenza agent into the virus with pandemic potency. Quickness and width of human influenza spreading by respiratory way among sensitive population are determined by properties of the agent and its high concentration in the small liquid drops. The particles of 4-40 μm diameters are mainly accumulated on the mucosa of the upper respiratory tract. Infectious dose of the virus is provided if tens to thousands infectious particles are contained in one drop of aerosol. It relates to both natural and postvaccinal infection [11, 13]. Therefore, in case of development of local or global epidemic we are talking of highly productive virus infection, characterized by accumulation of large amount of valuable virus' in upper respiratory tract. Now we will use these positions to overview what changes of avian influenza agent's properties are necessary for realizing the possible appearance of the virus with pandemic potency.

Four main stages of AIV changes must be realized in order the virus to become "human" (table 2). First, the avian influenza has to "learn" to infect the epithelial cells of human nasopharynx and trachea effectively. Second the avian influenza has to gain an ability to high level accumulation of its structural and non-structural components in the epithelial cells. At the same time it is necessary that high level of virus reproduction in the cells would be accompanied by the formation of the valuable virus particles capable to infect the cells of the upper respiratory tract of other people. Then, by natural selection highly pathogenic mutants must be adapted to the human population and formation of genetically stable infectious agent with new properties -

possible pandemic virus – must occur.

4.1. Acquisition by avian influenza the property of efficient absorption and penetration into cells of human respiratory tract. The first stage of cell infection starts from the virion adsorption, and the possibility of cell infection depends on the success of its realization. Adsorption is a highly specific process for influenza virus. Its specificity is based on the presence in virus hemagglutinin (HA) certain amino acid consequences – viral receptors. Recognition of the specific cell receptors and interaction with them is the function of viral receptors. Avian viruses (ducks, chickens, turkeys) are characterized by higher activity of binding the sialic receptors on the cell surface Sia (α2-3) Gal while human influenza viruses – with Sia (α2-6) Gal. The last type of receptors predominates on the human cells of respiratory tract. Both types of receptors (α2-3 и α2-6) are contained in the trachea cells of pigs and quails. Due to this ability, the animals listed can support the replication of both human and avian influenza viruses and to serve as a "mixing container" for their genetic interaction [14]. It was showed recently that human conjuctiva cells mainly contain the receptors α2-3. The appearance of zoonotic conjuctivits in human is explained by ocular tropism of AIV [15].

Chemically receptors of influenza virus are glycoproteins. Their protein part is coded by the virus genome and depends on the HA variability. Carbohydrate part is not coded by the virus genome. It determines not specificity, but affinity of the virus' receptors. Its structure depends on the host cells, namely on the including of carbohydrates of the cell membrane into the HA of influenza virus during reproduction. Several cycles of reproduction in intermediate host (pigs and probably quails) are considered desirable and even necessary condition during the formation of population of highly infective avian influenza virus in humans. Virus HA has to acquire a partial similarity with the surface carbohydrates of human influenza virus as a result of reproduction of avian virus in organism of intermediate hosts. An important property, determining its ability to begin the early stages of reproduction in the cells of human upper respiratory tract, has to appear in foreign virus. The fact attracts attention that this property of AIV doesn't even appear after replication in human lungs. This becomes understandable if to take into account that the alveolar epithelium differs substantially from the epithelium of trachea and nasopharynx.

During penetrating the virus into the cell, following the absorption, fusion membranes of cytoplasm and virus takes place. After that the inner virus' structures appear in the cytoplasm of the infected cells. Virus uncoating occurs that is partial virion deprotenization and releasing of viral nucleoprotein. Thus the conditions for viral genome transcription and replication by means of its polymerase (transcriptase) complex are created.

The process of viral uncoating like initial stages of specific absorption, is realized by

means of viral HA. The former has to be two polypeptides (HA1, HA2), that are produced represented by after proteolytic cleavage of the initial HA0 molecule (4.3.). Therefore, for effective infection of the upper respiratory tract cells by avian influenza its hemagglutinin must obtain two properties. First ability to attach to the cell surface, that is essential for the specific adsorption and second – uncoating property, providing fast penetration of the nucleoprotein into the cell by means of fusion of viral envelope and plasma membrane. Combination of these functions can be achieve as a result of the genetic changes in the binding site of HA1 and fusion peptide of HA2 as well as and phenotypic changes of the carbohydrate component of HA.

Noteworthy, influenza virus, like other viruses, by means of endocytosis can penetrate not only into sensitive but also into genetically resistant cells thus avoiding the stage of specific absorption. However in this case the fast virus "uncoating" doesn't take place, therefore the virions remain in the pinocytosis vacuoles and are destroyed quickly by lysosomal enzymes [16]. In this case the infecting of the cell doesn't take place, but even in case it occurs, the number of virus infected cells will be low.

4.2. Acquisition by avian virus the ability to high level accumulation of its components in human upper respiratory tract cells. Next stages of influenza virus' reproduction after penetration into cell are based on typical reactions common with the cell metabolism. Independently from the initial sensitivity or resistance of the cell the other stages of virus reproduction – transcription and replication of the viral genome, translation of the mRNA, virus assembly and virus yield – can be successfully realized in both systems. It is possible to infect a cell by virus in the certain conditions, even in case it does not have a necessary receptor, for example, using the infectious viral nucleic acid or phenotypic viral mixture, containing the particles with full genome of one virus and envelope of another. The plasma membrane can be artificially destroyed followed by successful infecting of non-permissive cells thus ignoring the receptor incompatibility. In genetically resistant cells only one cycle of the viral reproduction takes place and the new viral progeny, like initial viral particles cannot infect non-permissive cells. Only this type of interaction between AIV and human cells is observed so far in the cases of human infection by zoonotic influenza, when human-to-human virus transmission doesn't happen. Mechanisms of viral interaction with cell population, including genetically resistant cells, are known enough [16, 17, 18].

There are two ways for acquisition by AIV the ability to high level accumulation in genetically resistant cells of the human upper respiratory tract. The potentially main way is a reassortation of the fragments of viral genomes, represented by core fragments of AIV and envelope of human influenza. The other way, although of low probability, but theoretically possible – is represented by adaptive mutations without gene reassortment.

First, we consider the first way. Creation of reassortant viruses is used widely for increasing the economic efficacy of manufacturing the inactivated influenza vaccines (IIV). So, all vaccine viral strains (type A) for IIV are prepared from core components of highly productive influenza virus A/PuertoRiko/8/34 (H1N1) and surface components of epidemically actual influenza viruses. High yield of the reassortant is provided for gene complex coding the core virion structure.

Temperature conditions, determining the activity of new virus' transcriptase complex, play the key role in possible acquisition by reassortant (human influenza virus with AIV envelope) the property of high yield in cells of human upper respiratory tract. Normal temperature of the human body is about 5°C lower then that in birds. In fact this difference is much more, since it should be compared with the temperature of target cells – the mucosa of human upper respiratory tract (cooled by inhaled air) and intestinal tract of birds. Optimal temperature for activity of the transcriptase complex (PB1, PB1-F2, PB2, PA) is lower for human influenza virus, then for AIV. Therefore, the reassortant containing the core components of human influenza virus must acquire the ability to efficient transcription and replication of viral genome that, in turn, leads to high level of virus' reproduction in cells of human trachea and nazopharynx.

Appearance of influenza virus reassortants is possible in any organism, where the simultaneous reproduction of two different related viruses, necessary for their genetic interaction in one cell, can be realized. Man and domestic animals can be such organisms at the same extent. Pigs, having the high susceptibility to human influenza virus and AIV, can serve as reservoir of both appearance and storage of shift-variants with new antigenic formulae. Therefore pigs are the most real intermediate hosts of the reassortant viruses with pandemic potency.

Another, almost unreal, way of AIV property changes is adaptive mutation without reassortation. It can be realized only after several passages of the virus during its reproduction in the conditions of natural selection. In vitro and in ova in laboratory it is easy to select the mutant clones with high ability to reproduction. As for the effective realization of the dangerous for human natural mutations, it is practically impossible to be produced in the organism of the birds. Since AIV doesn't circulate among people, the natural selection of avian virus mutants, having the good survival and reproductive properties in human trachea and nazopharynx cells, can't take place in human population. However the stabilization of these mutations is possible during AIV reproduction in the organism of pigs. In this case two stages of changes of AIV properties mentioned above (see 4.1 and 4.2) will take place simultaneously. It is clear that such combination of changes of the virus may relate to reassortants developing in pigs.

4.3. Releasing of the highly infectious virus from infected cells of human upper

respiratory tract. Presence in the virion of the sufficient amount of cleaved HA is necessary to guarantee its infectivity. The increase in the virion of the number at cleaved molecules of HA leads to the strengthening of infectivity. Molecular mechanism of high infectivity is concerned with the content of the numerous sequences of amino acids in the site of HA cleavage. The presence of such mutations provides to the virus high infectious activity thus increasing its pathogenicity. HA of the highly pathogenic AIV (H5, H7) has acquired the property to be cleaved by different proteases as a result of spontaneous mutations during natural reproduction in avian organism. This relates to trypsin-like proteases present in the cells of the human respiratory tract and avian intestine and ubiquitous furin-like proteases present in the various tissues [1, 2, 9]. Such spontaneous mutations can be considered as a cause, randomly associated with the beginning of AIV adaptation to humans.

The rate of the HA proteolytic cleavage is strongly enhanced in the highly pathogenic AIV isolates. The rate of activation of hydrophobic fusion peptide, present in the site of cleavage of the HA, is also increased. It leads to strengthening of the virus' ability to the penetration into cell, specifically accelerating the stage of uncoating via the fusion of HA with the lipid bilayer cell of membrane. However these changes are not enough for AIV to infect the cells of the human upper respiratory tract even with a low efficacy. For that propose, the combination of two functions is necessary in HA– attachment and uncoating (see 4.1.).

4.4. Fixation of mutations linked to the high Pathogenicity in reassortants or adapted virus in human population, formation the genetically stable infectious agent – possible pandemic virus. Formation of pandemic virus in human population takes about 1-2 years. Process is begun with a moment of exposure the first local cases of human-to-human transmission of new subtype influenza virus A. Natural selection of the virus takes place as a result of infectious agent spreading among population. Selection of the new mutations, with a frequency about 10^{-6}, goes on in the direction of fixing the genetic properties of the pathogen, caused the highly contagiuos acute disease in the conditions of the active air-borne transmission of the agent among people. This process is finished by the formation of the new genetically stable virus – the possible pandemic agent.

Discussion and Conclusion

Due to its peculiarities, influenza infection can be poorly, if any, controlled. Next pandemic that was not observed for almost 40 years presents a possible threat. Strong genetic evidence obtained in the past years which support "avian" origin of the previous pandemic viruses (H1, H2, H3) and progressive worldwide influenza spread among wild and domestic

birds with sporadic cases of the most severe zoonotic avian influenza in humans are the facts allowing many leading world specialists, including WHO experts, suggest that the globe have entered the period of potential pandemic which will be caused by highly pathogenic AIV modified under natural conditions**.**. This opinion is expressed over already 9 years. However, during this comparatively long period, avian influenza morbidity among humans remained extremely low and no additional facts indicating inevitability of pandemic emergence within the next few years are available so far [4, 6].

It is important to know what type of variations in avian influenza agent should appear to determine its possible transformation into a pandemic virus, what stages and mechanisms could be responsible for these changes. We made an attempt to assess these aspects. Our considerations were based on the evident statement that even local epidemic caused by modified AIV developed in human nasopharyngeal and trachea cells will obligatory produce highly productive, not abortive, autonomous viral infection necessary for active air-borne human-to-human transmission of the virus. This efficient infection is characterized by formation of large quantities of mature infectious virus and is thoroughly studied using previously cell's cultures [17, 18]. In conditions of experimental production, high concentrations of human influenza virus**,** adapted to chicken embryos, may be accumulated in mammalian primary and continuous cell lines [19]. Addition of trypsin to cell culture medium is necessary to form mature infectious progeny of influenza virus previously adapted to chicken embryos. Trypsin compensates absence of necessary proteases in mammalian cells providing cleavage of influenza virus HA into two polypeptides – HA1 and HA2, which is important for manifestation of infectious properties of virions.

Analysis of scientific publications shows that transformation of AIV into the virus presenting **a** global danger to humans will require at least 4 basic stages to ensure significant variations of its properties.

First, AIV should acquire properties of effective absorption and penetration into human nasopharyngeal and trachea cells**.** To achieve effective infection of cells HA of AIV should combine two functions: that of attachment necessary for specific absorption, and that of uncoating necessary to provide rapid penetration of nucleoprotein inside a cell genetically resistant to the virus. These functions play the key role in overcoming species resistance of cells. They can be acquired only as a result of mutational changes, on the one hand, in the HA1 site responsible for attachment of the virus to the cell surface, and on the other hand, in the fusion peptide site of HA2 responsible for rapid penetration of viral nucleoprotein into cytoplasm. Effective virus infection of cells is possible only in case of successful preliminary HA cleavage into HA1 and HA2 occurring during virus maturation. It means that**,** correlation exists between

initial (the 1st) and further (the 3rd) stages of changes of AIV properties with no necessary stage-by-stage sequence of variations (see table 2). Affinity of viral receptors is significantly enhanced due to inclusion of host cell carbohydrates into HA composition. Since carbohydrates are not encoded by viral genome, they can not be inherited. Each cycle of viral reproduction in a new cell system mediates changes in viral receptors composition depending on plasma membrane forming the virion's envelope during their budding. Carbohydrates transferred to virions from human, swine or other mammalian cells will help to overcome species resistance of human organism to avian virus.

The second stage is acquiring by changed AIV ability to high-level accumulation of its components in human nasopharyngeal and trachea cells. This phenomenon may happen by means of reassortment of genome fragments of closely related viruses – human influenza virus and AIV or as a result of adaptive mutations (in transcriptase complex genes) of the avian virus. Practical realization of the first route seems to be more probable comparing with the second one.

Third, mature virions should be also characterized by high infectious properties and capability of replication in these cells during passages. This should occur due to variations in the very structure of avian HA allowing its large molecule to acquire ability to be split by trypsin-like proteases of human cells into two subunits. The cleavage occurs at the last stage of virus replication (the stage of assembling with participation of virus neuraminidase). Cleavage is necessary to form infectious virus capable to specific attachment and effective penetration into a cell. Currently we should take into account that HA of avian virus of H5N1 subtype has already acquired genetically inherited property to be cleaved by different human cells proteases. This property is associated with appearance of multiple sequences of basic amino acids in its cleavage site [2]. However high infectious capacity of such AIV remains notable only in birds being its natural hosts and does not manifest itself in humans. Why do certain people nevertheless, although rarely, are attacked by avian influenza, what phenomena mediate AIV selectivity for small part of human society? It appears to be associated not only with high infectious dose but also with some kind of specific failure of species immunity against AIV in affected persons. Clarification of the nature of this phenomenon may help to recognize mechanisms responsible for pronounced genetic resistance to AIV in humans and for its natural overcoming during zoonotic influenza.

As a result of the last, fourth, stage, which is necessary to change properties of a pandemic reference virus, new pathogen characteristics should be fixed in human population. Formation of a genetically stable and potentially dangerous virus will be realized via natural selection in case of its air-borne human-to-human transmission. Trends of selection include enhancement and fixing of pathogenic virus' ability to high-level replication in cells of human

upper respiratory tract**.** According to the new WHO classification [2] it corresponds with phases 4 and 5 of the pandemic threat period. Gradual with limited human-to-human virus transmission, adaptation of infectious agent to human organism involving initially restricted and further large groups of human population is supposed. Pandemic period (phase 6) starts with sharp increase of infection spread sustained at high levels among general population in different countries.

High AIV pathogenicity based on determination of main conditions mediating pandemic development (see 3) is not obligatory for neither possible appearance of pandemic virus nor even for acceleration of this process. Virus should be only pathogenic enough in order to cause acute form of the disease. In contrast, in natural conditions viruses of low pathogenicity have more possibilities for changing their properties including genetic interaction with other closely related viruses.

Increase of virus infectivity associated with HA proteolysis correlates with enhancement of its pathogenic properties. The more number of cells are infected and the greater is intensity of virus accumulation, the higher is pathogenicity of the infectious agent. Not only HA but also other AIV proteins serve as factors of pathogenicity [2, 12]. In particular, NS1 protein is capable to inhibit IFN-α,β system in antiviral defense, PB1-F2 protein causes formation of pores in mitochondria and induces monocytes and macrophages apoptosis that provides reduction in resistance to virus infection. AIV neuraminidase promotes virus penetration into a cell, its release from the surface of infected cell and thereby dissemination of the virus within the organism. M2 protein regulates pH in the process of uncoating of influenza virus in endosomes and in Golgi apparatus thus enhancing efficacy of virus reproduction. Proteins of transcriptase complex (PB1, PB1-F2, PB2 and PA) are responsible for intensity of AIV genome transcription and replication and hence of efficacy of accumulation of the virus.

As it follows from the presented discussion, birds are not suitable objects for realization of above indicated stages of variations of AIV properties. In case of preliminary introduction of avian virus into intermediate hosts such as swine, this process is alleviated. During the passages a jump in changing of properties of an infectious agent may occur manifesting in simultaneous instant realization of a number of stages with formation of a new virus potentially able to effective adsorption, penetration into cells of human upper respiratory tract and accumulation there resulting in formation of infectious virus.

Of course, this jump will not guarantee obligatory successful adaptation of the changed AIV to a human organism. Further progress of this process may be interrupted any time as it was, for instance, in 1976 during epidemic outbreak of “swine” influenza H1N1 virus among military recruits in the USA. That time the virus rapidly disappeared out of human population because human-to-human transmission was possible only during 4-7 passages [11, 13].

Interruption of circulation of a pandemic dangerous virus was undoubtedly caused by active sanitary-anti-epidemic measures apparently including mass specific immune prophylaxis of population using new urgently produced vaccine based on epidemically actual strains.

Thus, process of changing of AIV properties with tendency to possible transformation into a pandemic virus is characterized both by jump-like and gradual steps depending on the combination of many factors. There is no any evidence allowing confirm current introduction of avian virus into human population. Like in the previous years, cases of avian influenza among humans are extremely rare. Rates of avian infection among humans account for approximately 1-2 cases per 1 mln of people who live in regions affected by influenza epizootics. As before, no human-to-human transmission of the virus is available. Bold prognosis made by a number of leading specialists that in 2006 mankind will be face to pandemic with catastrophic consequences has not already been justified. It is clear now that pandemic will not be been in 2007 and obviously in 2008. Highly pathogenic influenza avian virus A (H5N1) actually has not been changed since it was isolated in 1997. Today only one of the 4 basic stages of AIV changes of properties necessary for potential originating from it of a pandemic virus has been accidentally realized. Therefore, the probability of obligatory rapid emergence of globally dangerous virus is evidently overestimated and the world is not on the threshold of appearance of gigantic pandemic. Many years of pandemic threat may be called as inter-pandemic period. Though it will be obviously too optimistic to confirm that mankind is not approaching closer to the possible pandemic. Future emergence of the overwhelming epidemic caused by AIV with changed properties is more than probable. In order to minimize pandemic consequences and to organize effective measures to combat impending threat WHO Global Pandemic Plan and National Pandemic Preparedness Plans are available [9, 10]. Comparatively accurate prognosis of the future influenza pandemic can be made only after appearance of the first proved local influenza outbreaks when air-borne human-to-human transmission of the virus.

Table 1.

Epizootic caused by highly pathogenic subtypes of AIV during last years

Years	Viruses	Countries and parts of world
1997, 2003, 2004	H5NI	Hong Kong, Korea
2003	H7N7	Netherlands
2003, 2004	H7N2, H7N3, H5N2	USA
2004	H7N3, H7	Canada, Pakistan
2004, 2005	H5N2	Japan, Korea, China
2004-2006	H5N1	Asia, Europe and Africa (Austria, Azerbaijan, Albania, Afghanistan, Bosnia, Great Britain, Hungary, Vietnam, Germany, Greece, Georgia, Egypt, Zimbabwe, Indonesia, Iraq, Iran, Kazakhstan, Cambodia, Cameroon, China, Laos, Mongolia, Niger, Pakistan, Russia, Rumania, Thailand, Turkey, Ukraine, France, Hrvatska, Czechia, Switzerland, Sweden, Japan, et.al.)

Table 2.

The main stages and mechanisms of the AIV property changes necessary for appearance possible pandemic virus

Stages	Mechanisms of realization
1. The acquisition by AIV the property to the effective adsorption and penetration into the human upper respiratory cells	The combination in the AIV HA of the mutations of HA1 and HA2, fulfilling the attaching and uncoating functions correspondingly. The increase of the HA of AIV affinity during specific adsorption due to including into it the carbohydrate component of the host cells (human, pig or other mammals)
2. The acquisition by AIV the property to the high-level accumulation of its components in the human upper respiratory cells	The appearance of the reassortants, consisting of the HIV core and AIV envelope, during simultaneous replication of two viruses. The primary adaptation by AIV in the organism of the intermediate host.
3. The obligatory formation of full virions, able to transmit by air-borne way from human to human, into the infected cells of the human respiratory tract	The mutations in the HA of the AIV. The large molecule of HA acquires ability for effective cleavage into two subunits (HA1, HA2) by proteases of cells epithelium of human respiratory tract.
4. The fixation of dangerous mutations of the reassortants or adapted viruses in the human population and formation the stable infectious agent – possible pandemic virus	The natural mutations selection of the reassortants or adapted viruses by way of serial continuous passages in human population serving as a subject to air-borne pathogen transmission.

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