Evolution of equine influenza virus between epizootics

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Abstract. New field strains of equid influenza virus isolated in different countries belong to independent branches in the Florida lineage, clade 2 – European and Asian. Asian viral isolates were akin to American evolutionary ancestors. Recently, there were local outbreaks of the disease with atypical symptoms among livestock. The initial diagnosis often meant a non-infectious problem. Throughout the year, horses of several equestrian bases periodically had a respiratory disease, which was characterized by a chronic course and low fever. A similar pathology in horses is described as such a chronic obstructive pulmonary disease. Sometimes the clinical picture comprised diarrhea and urticaria. In our research, several H3N8 equid influenza viral strains were isolated from the sick horses. Phenetic method of evolutionary relationships revealed their short tree distance to the equid influenza virus “A/eq/China/Ulumuqi/2015 (H3N8)” (nucleotide identity 98.43%) and viruses isolated from horses and camels in 2011-2013 in the southern regions of Kazakhstan and Mongolia (nucleotide identity 98.17%). The results supplement the data obtained by other authors characterizing the evolution and expansion of the H3N8 equid influenza virus in different geographical areas. This may allow improving the tools of diagnosis and prevention of infection.

1. Background

The equid influenza virus was first identified by O.Tumova and N. Sovinova in 1956. The causative agent was designated as equid influenza virus subtype 1 (H7N7). These findings were able to explain the etiology of acute mass equine disease, described previously as catarrh of the upper airways or Hoppegarten cough. In 1963, a fundamentally different subtype of two influenza virus H3N8 was discovered in the United States [1].

On the territory of the USSR, strains of the equid influenza virus of both types were first isolated by K.P. Yurov and N.N. Kryukov during the outbreaks of 1968-1974. The disease occurred in the border areas (railway border Chop point crossing Ukraine - Hungary). Epizootic was characterized by the fact that both viruses subtypes H7N7 and H3N8 were at the same time. The elimination of the first subtype of the virus finished at the end of the 70s. The last USSR epizootic caused by the virus of the first subtype (H7N7) was observed in high-mountain pastures horses in Kyrgyzstan in 1974. Horses had a multi-day fever, severe dry cough, and conjunctivitis. The mortality was 10-12%. At the autopsy, banded hemorrhages in the trachea, acute pulmonary edema, and focal degeneration of the heart muscle were observed. Because of the high isolation of mountain pastures, it can be assumed that the H7N7 virus was introduced into the herd of horses by migratory birds. Now only the virus of subtype two (H3N8) is in active circulation. Isolated strains were deposited at the WHO Regional Center Influenza [2].
The frequency of H3N8 outbreaks is observed with an interval of 10-12 years, sometimes 5-6 years. The causative agent is characterized by antigenic variability. The antigenic drift of the equid influenza virus H3N8 was registered in France in 1979 (strain A/equine/Fontainebleau/79) and in USA in 1980-1981 [3]. It was determined the beginning of an epizootic, during which it became clear that no commercial vaccine ensured the formation of sufficiently intense immunity to a new variant of the virus. A new strain was the ancestor of the Kentucky-Argentina lineage. Similar cases were recorded later in 1984-1985 in Europe and our country. In various regions of the USSR, 60–70% of horses immunized with vaccines against equid influenza were sick. All the USSR strains of the equid influenza virus were isolated pre-divergence in 1970-1988, and, thus, they do not belong to genetic lineages [2].

In 1989, a major epizootic of equid influenza was observed in Europe. Antigenic and genetic analyses of the virus isolated in the UK (A/eq/Suffolk/89) showed that the pathogen is significantly different from the strain (A/eq/Fontainebleau/79), which preceded it in Europe. Subsequently, similar strains were isolated in Europe and America.

Genetic analysis of the influenza virus strains isolated at this time showed that they can be divided into two groups (genotypes) – Eurasian (early European) and American. This division is based on the geographical origin of the isolated viruses. Moreover, the virus isolated in the United States may be of European origin and vice versa. The functional significance of this phylogenetic division was considered in a study of hemagglutinin (HA) using polyclonal and monoclonal antibodies. As a result, differences in the external virus antigens characteristic of each group were established, which can lead to insufficient cross-protection during vaccination [3].

Over the past 20 years, the equine influenza virus of the subtype two of European and American origin has spread throughout the world.

In recent years, the equine influenza virus has spread widely in Asian countries. Severe epizootics of equid influenza were observed in Mongolia and China. At the same time, approximately 80% of horses get sick, of which 20% were fatal. A similar outbreak of equid disease occurred in subsequent years, but no deaths were observed. Virus strains isolated at this time showed weak reactivity with antiserum to known strains A/equine/H3N8 of the American and Eurasian genotypes. Nucleotide analysis showed that birds became the source of infection, from which the viral agent spread to horses [4].

Pu J. et al, 2009 analyzed the antigenic composition of six strains of the virus (H3N8) isolated from domestic ducks during 2004-2005 in northern China. A phylogenetic study of these isolates showed their evolutionary relationship to strain A/equine/Jilin/89 (H3N8), which in the late 80s caused an outbreak of influenza infection among horses in the northern regions of China. This confirms the participation of birds in the spread of the equid influenza virus. In the period 2004-2006, two strains of the H3N8 influenza virus from pigs were isolated in the central part of China. Analyzing the amino acid composition, it was found that the isolates belong to the equid influenza virus (H3N8) of the Eurasian genotype, distinct in the early 90s [5].

Qi T. et al, 2010 observed an outbreak of equid influenza in 2007 in China. Accordance in results of a genetic analysis of the isolates (for example, A/equine/Xinjiang /5/2007), they were related to the American strains of equid influenza virus and not related to equid influenza virus isolated from pigs in 2004-2005 in China [6].

Bryant N.A. et al, 2009 conducted a phylogenetic study of influenza virus strains in North America and Europe during 2006-2007. As a result of these studies, authors were able to identify equid influenza virus strains of both Eurasian and American genotypes. Besides, among the studied strains, it was possible to identify equid influenza strains (H3N8) of the previously named Eurasian lineage A/eq/Switzerland/P112/07 and A/eq/Lincolnshire/1/06, which have caused recent epizootics in Asian countries [7]. Now the Eurasian lineage is two branches of Florida lineage, clade 2.

Then in 2007-2008 the global panzootic of equid influenza occurred, including on the territory of the Russian Federation. We isolated and identified strain “A/equine/Bitsa/2007 (H3N8)” of the Florida clade 2, based on which the inactivated vaccine is currently produced by company Biok Co. (Kursk, Russia) [8].
In total epizootics of influenza in horses of recent years in Croatia (2004), Italy (2005), Russia (2007), Egypt (2008), Australia (2008), Kazakhstan (2012), China (2015), etc. emphasize the ease with which this infection can spread among susceptible animals as a result of international horse movement. Based on this, the OIE Guidelines recommend that flu-free importers require compulsory vaccination of all imported horses against this infection [9].

Of interest is the fact that there have been reports of the spread of the H3N8 virus, which causes an acute respiratory infection in the greyhounds [10]. Molecular genetic analysis of the strain (A/canine/Florida/2004-2005) established its identity (96%) to the second subtype of equid influenza virus, which indicates direct transmission of the virus from horses to dogs without recombination with other strains. A similar viral disease of dogs was observed in 9 countries in 2004-2006. Moreover, in some cases, the viral infection of dogs ended in death with hemorrhages in the lungs, mediastinum, and blood build-up in the pleural cavity. Serological monitoring conducted in Florida and New York among dogs from shelters, veterinary clinics, and kennels revealed seropositive animals for equid influenza virus (H3N8) in 25 districts [11]. This suggests that the spread of the influenza virus among dogs is not limited to a specific group of animals, but maybe massive. According to some authors such an expansion of the host equid influenza virus (H3N8) associated with mutations in hemagglutinin may raise an issue of the possibility of humans’ infection [12].

The very long interepizootic intervals of equine influenza are due to the presence of hidden reservoirs of the persistent virus. Targeted research in this regard is necessary to improve the means and methods of specific prevention of the disease. This study aimed to monitor and control the circulation of H3N8 equine influenza virus between epizootics in the Russian Federation.

2. Materials and methods

2.1 Viruses
We used reference strains of equine influenza virus - “A/equine/Prague/56” (H7N7) and “A/equine/Miami/63” (H3N8) and new strains were detected in 2011-2018 in Russia - “A/equine/Odintsovo/2011” (H3N8) and “A/equine/Krasnogorsk/2018” (H3N8).

2.2 Chicken embryos
Viral isolates were replicated in 9 - 10 daily SPF chicken embryos (“VALO-SPF”, Germany).

2.3 RT-PCR and nucleotide sequencing
RT-PCR for hemagglutinin (HA) genes for equine influenza virus H3N8 was carried out by the method described previously [8]. The nucleotide sequencing was performed at Syntol Co (Russia).

2.4 Phylogenetic analysis
Local and multiple alignments of nucleotide sequences were carried out with the tools of the BLAST and ClustalΩ software packages. The dendrogram was reconstructed in the MEGA 7 software by the neighbors-joining algorithm. The reliability of the evolutionary relationship reconstruction was accomplished by Bootstrap analysis based on 1000 replicates.

2.5 Serological testing
Antibodies to the equine influenza virus H3N8 in the sera of convalescent horses were tested by HI and ELISA as described previously [13].

3. Results and discussion
On farms of various types, the asthma-like chronic equine disease is often observed, which is characterized by signs of chronic alveolar emphysema, pulmonary hypertension, and chronic pulmonary heart, leading to progressive obstructive pulmonary ventilation and gas exchange. By analogy with
medical practice, it’s diagnosed as a chronic obstructive pulmonary disease (COPD) [14]. Traditionally, COPD is interpreted as a non-infectious problem caused by inaccuracies in captivity and feeding.

Under the medical literature, the factor of viral infection is very considerable in the anamnesis of the disease. The part of viruses in the COPD reactivation is 30–56%. Most often, rhinovirus (26–36% of all infectious agents), RSV (29%), influenza virus (16%), coronavirus (11%) are detected. In humans of COPD remission, the range is much lower: rhinovirus - in 7.3% of cases, RSV - 16.2%, coronavirus - 5.9%. At the same time, the recurrence of the disease was more likely in humans with persistent respiratory viruses [15].

In our practice, we tried to find hidden reservoirs of equid influenza H3N8 virus after the last epizootic it caused in Russia in 2007-2008. We turned our attention to horses with long-term asthmatic diseases and collected nasopharyngeal secretions from them for retrieval of the influenza virus. The results are presented below.

Throughout the season of minor respiratory diseases of sports horses in October 2011, the equid influenza virus was identified in their nasopharyngeal secretions. A new variant was designated as “A/equine/Odintsovo/2011” (H3N8). Revision given the evolving context and ecological-geographical relationships was accomplished by phylogenetic analysis. The results showed relation in a closely distant way to some viruses isolated from horses in 2010 - 2011 in Germany: “A/equine/ Bokel/1/11” and “A/equine/Hamburg/10” (nucleotide identities of 99.99% and 99,73%). The affiliation of German strains to the genetic lineage remained unclear for some time. Early it was meant that they belong to the emerging Eurasian lineage or intermediate between that and Florida clade 2. Now German isolates are assigned to the Florida lineage of the European sublineage 2 of the American genotype.

During 2018, the respiratory disease was periodically observed in the horses of the sports base, which was diagnosed as “chronic obstructive pulmonary disease” - COPD. A horse was chosen with symptoms of relapsing fever, shortness of breath, periodic hacking cough, and mucopurulent nasal discharge. From the collected samples a virus designated as “A/equine/Krasnogorsk/2018” (H3N8), was isolated in chicken embryos. The etiological role of the influenza virus was confirmed by a retrospective serological testing in HI and ELISA. Multiple alignments of the nucleotide sequences of the HA segment of isolate “A/equine/Krasnogorsk/2018” (H3N8) with those of other strains and dendrogram reconstruction showed its short tree distance to the virus “A/equine/China/Ulumuqi/2015” (H3N8) (98.43%) and also to strains isolated from horses and camels in 2011-2013 in the southern regions of Kazakhstan and Mongolia (nucleotide identity 98.17%). The new isolate belongs to the Florida lineage of the Asian sublineage 2 of the American genotype. The results of dendrogram reconstruction are demonstrated in figure 1.

4. Conclusion
Equid influenza usually occurs as an outbreak (or epizootic) of acute respiratory infections. The H3N8 virus, the causative agent of equid influenza now belongs to the Florida lineage, FC1 and FC2 clusters. Phylogenetic analysis of the full-sized genome concluded that FC1 and FC2 are divided and diverging clades, with no reassortment between them. In this study, we were identified the equid influenza virus strains isolated from Russian horses between epizootics. Last of them, typical, designated “A/equine/Krasnogorsk/2018 (H3N8)”, has been used to sequence partial HA genome segments. A new isolate has akin to strain “A/equine/China/Ulumuqi/2015” (H3N8) (98.43%) and viruses 2011-2013 isolated from Kazakh and Mongolian horses and camels (98.1%). Reassortment of influenza A viral genes causes antigenic drift or shift in the polypeptide structure of hemagglutinin, less commonly neuraminidase. Such changes may do not allow the development of effective vaccines. Therefore, the monitor of equine influenza virus between epizootics and search for new candidate vaccine strains is important for science and practice.
Figure 1. Dendrogram.

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