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# MODERN DIAGNOSTICS AND VACCINATION OF EQUINE INFLUENZA

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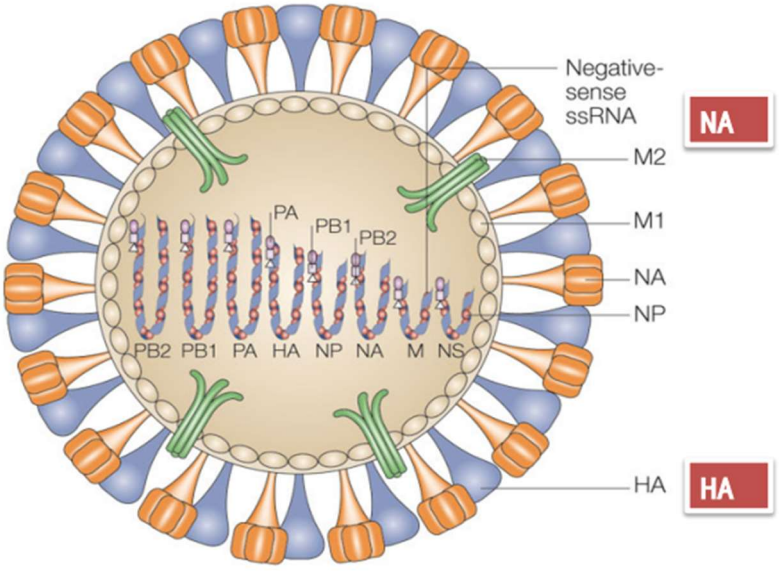
**Annotation.** This article presents the main methods for diagnosing and vaccinating viral diseases of horses.

**Key words:** equine influenza virus, diagnostics, vaccination, whole-virion vaccines, subunit vaccines, attenuated vaccines, vector-based vaccines, recombinant vaccines, reverse genetics methods.

Equine influenza (*Equine Influenza)* is an infectious, contagious disease of the equine family, characterized by catarrh of the upper respiratory tract, general depression, short-term fever and dry, painful cough, in severe cases pneumonia develops. Scheme of the influenza virion. (Picture 1)




Picture 1

Shell protein: **HA,NA**
Matrix protein: **M1,M2**
Polymerase complexes: **PB2,PB1,PA**
Nucleoprotein:**NP**
Non-structural protein:**NS1,NS2**

Session 7_Laboratory Diagnostic Techniques and Vaccine forEquine Influenza-Wei Guo

Based on the antigenic differences between the two surface glycoproteins (*HA and NA*), two subtypes of equine influenza virus have been identified.

The first strain of the equine influenza virus *A/equine/Prague/56* was isolated in Czechoslovakia in 1956 and had the antigenic formula *H7N7*. The *A/H3N8* subtype is thought to be due to a mutation in the avian influenza virus. The strain *"A/equine/Miami/63"* subtype *A/H3N8* was first isolated in Miami in 1963.

Equine influenza is a highly contagious disease characterized by a tendency to rapidly spread and a high incidence among susceptible livestock. The causative agent of equine influenza is an *RNA*-containing virus belonging to the *Orthomyxoviridae* family, genus *Influenzavirus.*

Infectious diseases cause significant damage to modern horse breeding. After infection, the influenza virus very quickly penetrates the epithelial cells of the respiratory tract of the horse, and causes their damage and subsequent death. The main clinical signs of influenza infection include fever (temperature rises to 41°C), cough, and mucopurulent discharge from the nasal cavity. Also, sick horses lose their appetite and become lethargic. If left untreated, fever can last up to 10-14 days, and sick animals can lose a lot of weight. Cough and mucopurulent discharge from the nasal cavity appear on the second day, and may persist for 3 weeks. Complications arising during the course of the disease - bacterial pleuropneumonia, pericarditis, and laminitis can lead to the appearance of additional clinical signs and a more severe course of the disease, and sometimes even death.

Timely diagnosis greatly helps to identify diseases at an early stage.

Currently, the isolated virus is identified using *PCR* and serological reactions: *ELISA*, *RIF*, *RTGAd*, *RN*. There are various methods for laboratory diagnosis of equine influenza virus: detection of the virus per se (electronic microscopy), detection and identification of viruses by means of cells interacting with it (light microscopy, culture method), virological methods (detection of the virus antigen using *ELISA, MFA*, and others.), detection of *DNA* of an infectious agent (polymerase chain reaction, dot hybridization method), serological diagnostic methods (detection of specific antiherpetic antibodies).

To identify all clinically significant subtypes, commercial kits are used: *Equine Flu H3N8 & H7N7*, *Real Time PCR Detection Kit Equine Influenza* A virus (*H3N8 & H7N7*) and others.

Vaccination against equine influenza, along with quarantine and restrictive measures, is one of the main tools for disease control. The main goal of vaccination is to reduce the manifestation of clinical symptoms of the disease and, as a result, improve the welfare of animals, which helps to reduce the period of convalescence and reduce the likelihood of developing secondary infections. In addition, vaccination reduces the release of field virus into the environment and thus prevents the spread of infection. Since the effectiveness of vaccination against equine influenza depends on the degree of antigenic homology between vaccine and circulating virus strains, vaccines should include topical circulating *EIV* strains recommended by the *OIE* (*International Epizootic Office, World Organization for Animal Health*, France). Since 2010, the *OIE* has recommended that representative strains of *HHF* subtype *H3N8* of the Florida clade 1 (*South Africa/03 or Ohio/03*) and Florida clade 2 (*Richmond/1/07*) sublines be included in equine influenza vaccines. The inclusion of strains of the subtype *H7N7* and *H3N8* (European lineage) is optional. The review presents up-to-date data on the types of vaccines used in practical horse breeding. Among them are inactivated whole-virion, subunit, as well as live attenuated and vector vaccines. In addition, data on the development of experimental vaccines against equine influenza obtained using modern genetic engineering methods are presented. The technology of reverse genetics of influenza viruses is considered, which makes it possible to improve the process of obtaining prototype viral strains for inactivated and live attenuated vaccines. The method of reverse genetics allows not only to obtain reassortant influenza viruses with the required antigenic

properties and reduced virulence, but also makes it possible to modify them, following changes in the antigenic properties of circulating field strains.

Vaccines against equine influenza that are currently used in veterinary practice can be divided into three groups according to the manufacturing technology: inactivated whole virion/subunit, live attenuated and vector. (*Table 1*)

Table 1

| Name | Company manufacturer | Adjuvant | Antigen | EIV strains |
|---|---|---|---|---|
| Inactivated whole virion/subunit | | | | |
| DuvaxynTm IE Plus « | «Elanco» (USA) | Carbopol | Whole VHF virions | Newmarket/1/93 (H3N8) Suffolk/89 (H3N8) Prague/56 (H7N7) |
| Calvenza®-03 EIV | «Boehringer Ingelheim Animal Health» (Germany) | Carbopol | Whole VHF virions | EIV Newmarket/2/93 (H3N8) Kentucky/2/95 (H3N8) Oiho/03 (H3N8) |
| Equilis Prequenza (updated in 2013) | «MSD Animal Health» (USA) | ISCOM-Matrix | Whole VHF virions | Newmarket/2/93 (H3N8) South Africa/4/03 (H3N8) |
| Equilis Prequenza | «MSD Animal Health» (USA) | ISCOM-Matrix | Subunits HA | Prague/56 (H7N7) Newmarket/1/93 (H3N8) Newmarket/2/93 (H3N8) |
| EquipTM F | «Pfizer Ltd.» (США) | ISCOM | Subunits HA and NA | Newmarket/77 (H7N7) Borlänge/91 (H3N8) Kentucky/98 (H3N8) |
| Equine influenza vaccine inactivated polyvalent | «Kursk Biofactory» (Russia) | aluminum hydroxide | Whole EIV virions | Cambridge-63(H7N7) France-98(H3N8) |
| Live attenuated cold adapted | | | | |
| Flu Avert® I.N. | «Intervet/ScheringPlough Animal Health» (Netherlands) | No | Whole EIV virions | EIV Attenuated cold-adapted virus Kentucky/91 (H3N8) |
| Vector | | | | |
| PROTEQ FLU™ | «Merial Animal Health Ltd.» (France) | Carbomer | HA | Ohio/03 (H3N8) Newmarket/2/93 (H3N8) |
| PROTEQ FLU™ (updated in 2014) | «Merial Animal Health Ltd.» (France) | Carbomer | HA | Ohio/03 (H3N8) Richmond/1/07 (H3N8) |

Note. *EIV*, equine influenza virus; *HA*, hemagglutinin; *NA*, neuraminidase.

After a major outbreak of equine influenza caused by the *H3N8 EIV* subtype in Kazakhstan (2007), the first Kazakh live modified vaccine against equine influenza was developed in cooperation with the Research Institute of Influenza (*Influenza Research Institute*, *St. Petersburg*). Using the methods of classical genetic reassortment, a vaccine strain A/HK/Otar/6:2/2010 was obtained, carrying genes encoding surface proteins (*HA, NA*) of the wild strain *A/equine/Otar/764/2007* (*H3N8*, American line Florida , clade 2), and genes encoding internal proteins (PB2, PB1, PA, NP, M, NS) of the attenuation donor, the ca strain *A/Hong Kong*/1/68/162/35 (*H3N2*). The safety and efficacy of the vaccine has been studied in horses 221. Currently used in horse breeding farms in Kazakhstan

**Conclusions.** Thus, equine influenza is a highly contagious disease characterized by a tendency to spread rapidly and a high incidence among susceptible livestock. Its outbreaks can significantly affect the horse breeding industry.

The conducted studies show that the polymerase chain reaction *PCR* method has certain advantages for diagnosing equine influenza virus. This method is more practical, specific and sensitive for the detection of equine influenza virus and allows a diagnosis to be made within a few hours compared to other known laboratory methods.

Equine influenza vaccination is an effective tool to prevent the disease. The first generation of vaccines were inactivated whole-virion and subunit vaccines, which produce protective antibodies. Subsequently, a second generation of vaccines (live attenuated and vectored) has appeared that stimulates humoral and cellular immune responses and mimics the protective immune response that occurs during natural infection with equine influenza virus.