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VIRAL SAFETY ISSUES IN THE PRODUCTION AND MANUFACTURING OF HUMAN IMMUNOGLOBULIN PREPARATIONS FROM EQUINE PLASMA/SERUM

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The current Russian and foreign pharmacopoeias either do not provide any information about existing types of viral diseases in horses or do not present it in full. Data of modern domestic and foreign literature was used to prepare the most complete list of viruses that cause equine diseases including 36 infectious agents, 25 of which are pathogenic for humans, 13 of the 25 of which are widespread throughout Russia. Information is provided on the magnitudes of the disease incubation periods (which are most often within one month), the external clinical signs of these diseases (which can also be asymptomatic), and the maximum possible concentrations of viruses in the blood of horses with these diseases (which can reach 8 log conventional units/mL of blood). This information is offered for use in critical production stages of heterologous immunoglobulin drugs for medical use to assure viral safety.

Keywords: horse, viral infection, distribution area, virus concentration, equine immunoglobulin.

Various injectable drugs for medical use based on immunoglobulin and their $F(ab)_2$ fragments from horse blood plasma/serum are currently being developed and manufactured in the Russian Federation and abroad [1 – 3]:

–from especially hazardous viral diseases,

–from bacterial toxins,

–from snake and scorpion venoms.

The production and control of this blood plasma/serum and drug intermediates based on it should consider data on existing types of diseases in horses caused by viruses pathogenic for humans to minimize the risk of viral contamination. However, no such information in this area is given in the current *State Pharmacopoeia of the Russian Federation*, XIVth Ed. (SP RS XIV). Leading foreign regulators specify different numbers of viruses pathogenic for humans that cause diseases in horses. For example, recommendations of the European Medicines Agency (EMA) indicate 10 such diseases of 16 [4]; the *Japanese Pharmacopoeia*, 17th Ed. (JP 17), 7 [5].

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Therefore, the aim of the present work was to use data from the domestic and foreign scientific literature to compile a list of viruses, including information on diseases that they cause in people. This information is necessary for manufacturers of equine immunoglobulin drugs:

–to choose animals (blood donors);

–to control horse blood plasma/serum and drug intermediates;

–to choose model viruses for work to assure viral drug safety.

For this, information on the following areas was selected using the modern scientific literature:

1) viral disease vectors in horses that are pathogenic and nonpathogenic for humans;

2) the distribution area of viral diseases in horses;

3) the incubation period and clinical presentation of viral diseases in horses including the percent deaths;

4) maximum virus concentrations in horse blood with viral diseases.

Table 1 presents the results of the investigations.

Table 1 shows the following:

36 viral diseases have been recorded worldwide in horses (instead of the 16 noted in the EMA materials [6]);

TABLE 1. List of Infectious Diseases in Horses Caused by Viruses Pathogenic and Nonpathogenic for Humans

No.	Disease vector species and family	Infection distribution area	Infection incubation period	Maximum virus blood concentration	Clinical presentation of disease at the maximum	Maximum % lethality	Ref.
1	Eastern equine encephalomyelitis virus* Togaviridae	North, Central and South America	5 – 14 d	5.6 log PFU/mL**	Hyperthermia, anorexia, dizziness, ataxia, cramps, palsy, paralysis, disturbed motor coordination	50 – 90	6 – 9
2	Western equine encephalomyelitis virus* Togaviridae	North and South America	5 – 14 d	7.5 log PFU/mL**	Hyperthermia, anorexia, dizziness, ataxia, cramps, palsy, paralysis, disturbed motor coordination	40	6, 10, 11
3	Venezuelan equine encephalomyelitis virus* Togaviridae	Central and South America	1 – 5 d	7.4 log LD_{50}/mL	Hyperthermia, anorexia, dizziness, ataxia, cramps, palsy, paralysis, disturbed motor coordination	50	6, 12 – 14
4	Getah virus* Togaviridae	Europe and Australia	2 – 6 d	4.2 log TCD_{50}/mL	Fever, leg edema (usually hind extremities) and stiffness	0	15
5	Ross river virus* Togaviridae	South America, Australia, New Zealand	7 – 9 d	6.3 log LD_{50}/mL	Edema of distal leg parts, synovial exudate, petechial hemorrhages, submaxillary lymphadenopathy, transient hyperthermia, tachypnea, refusal to walk	0	16, 17
6	St. Louis encephalitis virus* Flaviviridae	North and South America	4 – 21 d	5.5 log PFU/mL**	Disturbed motor coordination, depression, flaccid paralysis of hind extremities	30	18, 19
7	Japanese B encephalitis virus* Flaviviridae	Tropical Asia, rarely in southern Europe	4 – 15 d	3.0 log genetic copies/mL (GC/mL)**	Hyperthermia, anorexia, dizziness, ataxia, cramps, palsy, paralysis, disturbed motor coordination, inflammation of mucous membranes	40	20, 21
8	West Nile fever virus* Flaviviridae	Whole world (more often in warm countries)	3 – 5 d	2.7 log PFU/mL	Anorexia, depression, neurological symptoms	57	22, 23
9	Tick-borne encephalitis, Central European encephalitis, Russian spring-summer encephalitis virus* Flaviviridae	Europe, Siberia, Far East	10 – 20 d	Live virus not observed	Symptomless course but sometimes anorexia, nervousness, ataxia, cramps and epileptic seizures, and hyperalgesia in the neck	0	24
10	Dengue virus* Flaviviridae	Tropical Africa, Asia, Australia	Not determined	Live virus not observed	Symptomless course	0	25, 26
11	Zika virus* Flaviviridae	Central and South America	Not determined	Live virus not observed	Symptomless course	0	25, 27
12	Vesicular stomatitis virus* Rhabdoviridae	Europe and Australia	3 – 7 d	Live virus not observed	Blisters, papules, erosion near mouth and hooves	0	28
13	Rabies virus* Rhabdoviridae	Whole world	Up to 5 months	Live virus not observed	Fever, limping, ataxia, paralysis, incontinence, tremor, depression, aggressiveness, cramps	100	29
14	Equine herpesvirus type 1 – 4* Herpesviridae	Europe, USA	Persistent infection	3 log GC/10^6 leukocytes = 3 log GC/mL (cell-associated with leukocytes)	Fever with depression, anorexia and eye emissions, abortions, myeloencephalopathy, neurological symptoms	50	30, 31

No.	Disease vector species and family	Infection distribution area	Infection incubation period	Maximum virus blood concentration	Clinical presentation of disease at the maximum	Maximum % lethality	Ref.
15	Equine morbilli virus, Hendra virus* Paramyxoviridae	Australia	3 – 16 d	2.5 log GC/mL** (cell-associated with leukocytes)	Edema of distal leg parts, synovial exudate, petechial hemorrhages, submaxillary lymphadenopathy, transient hyperthermia, tachypnea, refusal to walk	66	32, 33
16	Nipah virus, Nipah henipavirus* Paramyxoviridae	Malaysia, South Asia	7 – 14 d	4.16 log PFU/mL of plasma** and 7 log GC/mL** (cell-associated with leukocytes)	Fever, tachypnea, nasal emissions, neurological symptoms (ataxia, dizziness, loss of vision, tilt of head, clonic convulsions, incontinence)	75	34 – 36
17	Equine influenza virus* Orthomyxoviridae	Whole world	1 – 3 d	1.8 log PFU/mL**	Hyperthermia, cough, rhinorrhea, anorexia	Very rare	37
18	Borna disease virus* Bornaviridae	Germany	60 – 90 d	Live virus not observed**	Pronounced excitability, aggressiveness or apathy, dizziness, palsy, paralysis, drowsiness, stupor and coma	100	38
19	Reovirus types 1 – 3* Reoviridae	Whole world	3 – 14 d	5.2 log LD_{50}/mL (cell-associated with leukocytes)	Cough, rhinorrhea, diarrhea	Rare	39
20	Equine rotavirus* Reoviridae	Whole world	1 – 4 d	Live virus not observed	Diarrhea, apathy, anorexia, and bloated stomach in foals	Rare	40
21	Equine poxvirus* Poxviridae	Finland, Mongolia, Africa	5 – 10 d	2.5 log PFU/mL**	Fever, edema of scrotum and ventral edema, multifocal, solid, nodal skin damage, increased lymph nodes	0	37, 41
22	Equine adenovirus* Adenoviridae	Whole world	3 – 6 d	8 log GC/mL**	Cough, wheezing, conjunctivitis, fever, diarrhea	Rare	42
23	Encephalomyocarditis virus* Picornaviridae	Europe, America, Korea, China, Australia	1 d	1 log LD_{50}/mL**	Symptomless course	0	43, 44
24	Foot-and-mouth disease virus* Picornaviridae	Africa, South Asia, America and Europe	2 – 14 d	No information	Symptomless course	0	45
25	Equine coronavirus* Coronaviridae	Whole world	2 – 4 d	6.2 log GC/mL	Fever, anorexia, apathy, colic and diarrhea	27	46, 47
26	Louping-ill, Ovine encephalomyelitis, Infectious encephalomyelitis of sheep, Trembling-ill virus Flaviviridae	Great Britain, Bulgaria, Turkey, Norway, Iceland, Greece, Japan, Far East	6 – 18 d	6.7 LD_{50}/mL**	Fever, anorexia, apathy, colic and diarrhea	10	48
27	Equine hepacivirus Flaviviridae	Whole world	Persistent infection	6.6 log GC/mL	Chronic hepatitis with long symptomless course	Rare	49
28	Equine pegivirus Flaviviridae	Whole world	Persistent infection	6.6 log GC/mL	Chronic hepatitis with long symptomless course	Rare	50
29	Equine and bovine papilloma viruses Papillomaviridae	Whole world	11 – 32 d	6 log GC/mL (cell-associated with leukocytes)	Outgrowths on skin and gastrointestinal tract and bladder mucous	Rare	51, 52
30	Equine arteritis virus Arteriviridae	North and South America, Europe, Asia, Africa	2 – 14 d	5.3 log TCD_{50}/mL	Fever, depression, edema, conjunctivitis, nasal emissions, abortions	Rare	53

No.	Disease vector species and family	Infection distribution area	Infection incubation period	Maximum virus blood concentration	Clinical presentation of disease at the maximum	Maximum % lethality	Ref.
31	African horse sickness virus Reoviridae	Africa, Middle East, Mediterranean, partially Asia (India)	3 – 14 d	5.0 log TCD_{50}/mL	Mild form: fever, serious respiratory disorder, progressing wheezing; cardiac: fever; edema of eyelids, head, neck; heart failure; mixed; feverous	95	54
32	Equine rhinitis A/B virus Picornaviridae	Whole world	3 – 8 d	5.5 log GC/mL	Fever, anorexia and copious nasal emissions	Rare	55
33	Equine infectious anemia virus Retroviridae	Whole world	7 – 45 d	3.2 log TCD_{50}/mL	Weight loss, edema, intermittent fever	Rare	56
34	Equine foamy virus Retroviridae	Whole world	Persistent infection	2.9 log GC/10^6 leukocytes or GC/mL (cell-associated with leukocytes)	Symptomless course	Rare	57
35	Equine parvovirus-hepatitis, Theiler disease virus Parvoviridae	East and West Germany	Persistent infection	7.5 log GC/mL	Chronic hepatitis with long symptomless course; 1 – 2% of horses with symptoms: apathy, anorexia and jaundice, neurological signs	Rare	58, 59
36	Cytomegalovirus, Herpes virus 4 Herpesviridae	Whole world	10 – 20 d in swine	2.1 log TCD_{50}/mL**	Symptomless, sometimes ulcers on cornea, conjunctivitis, pharyngitis, fever and cough in foals	Rare	60, 61

Note.
* Virus pathogenic for humans;
** maximum value of parameter obtained in experiments on model animal species (mouse, rabbit or groundhog).
PFU, plaque-forming unit in cell culture;
GC, genetic copy;
LD_{50}, 50% lethal dose for mice;
TCD_{50}, 50% tissue cytopathogenic dose in cell culture.

69% of viral diseases in horses (25 of 36) are caused by vectors pathogenic for people (instead of 10 and 7 noted in materials of the EMA [4] and JP 17 [5]);

52% of viral diseases of horses (13 of 25) that are caused by vectors pathogenic for people have distribution areas in Russia;

61% of all viral diseases of horses (22 of 36) have distribution areas in Russia;

many viral diseases of horses have mainly the same or a different clinical presentation, including death; however, they all can progress in an unapparent form (symptomless);

the maximum possible concentration of several viruses in horse blood reaches 8 log conventional units (CU)/mL with clearly pronounced clinical symptoms of the corresponding infection usually observed;

the incubation period of most equine viral diseases (not considering persistent infections) varies over a broad range, from 1 to 90 d but mainly up to 1 month.

Also, many researchers note in their publications that, as a rule, a frequently observed symptomless course of most viral diseases in horses occurs with lower peak virus blood concentrations than those noted in the table.

Therefore, horses (blood donors) for manufacturing of drugs based on specific immunoglobulins must be acquired from institutional farms safe for the 36 infectious diseases, especially paying attention to those vectors that are pathogenic for humans (25 noted in Table 1) and have distribution areas including Russia (13 viruses: Getah, Japanese encephalitis, West Nile fever, tick-borne encephalitis, rabies, equine herpesvirus types 1 – 4, equine influenza, encephalomyocarditis, foot-and-mouth disease, reovirus types 1 – 3, equine rotavirus, equine adenovirus, equine coronavirus). However, one of these pathogens, rabies, is not distributed in horses in Russia because all animals of this species in Russia are vaccinated against it. The manufacturer of the drugs should obtain from this farm the corresponding veterinarian certificates and quarantine the acquired horses (before starting immunization with the corresponding antigen) for one month (considering information of Rossel'khoznadzor regarding viral infections in horses [7]).

It is also noteworthy that all species of viruses causing disease in horses span a broad spectrum of families (17), of which 12 (Togaviridae, Flaviviridae, Rhabdoviridae, Herpesviridae, Paramyxoviridae, Orthomyxoviridae, Bornaviridae, Reoviridae, Poxviridae, Adenoviridae, Picornaviridae, Coro-

naviridae) contain equine viruses that are pathogenic for humans. Therefore, any viruses convenient for the researchers, a minimum of three virus species from these families belonging to three types, i.e., nonenveloped, enveloped RNA-containing, and enveloped DNA-containing (e.g., adenovirus, influenza virus, and vaccinia virus, respectively), can be used to choose model virus species required to validate technologies with different mechanisms of inactivation/elimination of viral contaminants in various materials during manufacturing of drugs based on horse blood. Studies to validate, e.g., two technologies with different mechanisms (physical, enzymatic, chemical, immunological, etc.) of inactivation/elimination of viral agents that are mentioned in pharmacopoeial requirements of leading global regulators [5, 62, 63] must reduce the infectious activity of the model viruses by at least 8 log units (100 million times). Considering the maximum possible accumulation level of several viruses in horse blood with the corresponding viral infections [8 log genetic copies (GC)/mL, Table 1], this degree of reduction of the infectious activity would practically exclude the presence of viable pathogen in the blood plasma/serum of this animal species. Moreover, if it is considered that horses with high virus blood concentrations would be immediately rejected because of their clearly pronounced clinical presentation of the disease.

Thus, modern domestic and foreign literature was used to compile the most complete list of viruses causing diseases in horses that included 36 viruses, 25 of which were pathogenic for humans and 13 of the 25 of which were pathogenic for humans and distributed in Russia. This list also contained information on the magnitude of incubation periods of the disease that are most often within one month; on the external clinical symptoms of these diseases that can also progress without symptoms; and on the maximum possible virus blood concentrations in horses with these diseases that can reach 8 log CU/mL of blood for individual infectious agents.

The analyzed data could be used at various manufacturing stages of equine immunoglobulin drugs for medical use in the following areas:

acquisition by drug manufacturers of horses (blood donors) from the corresponding institutional farms, paying attention to the presence/absence of viral diseases encountered among horses in Russia, especially those caused by viruses pathogenic for humans;

control of horse blood plasma/serum (for their possible rejection) during their quarantine (in the acquisition stage) to check for the presence/absence of viruses pathogenic for humans that are distributed in Russia;

control of pools of immune blood plasma/serum from immunized horses to check for the presence/absence of viruses pathogenic for humans encountered in Russia;

choice of model viruses from families in which disease vectors in horses that are pathogenic for humans are found to validate technologies for inactivation/elimination of the viruses used in the drug manufacturing process;

control of drug intermediates from the moment of filling the primary packaging to check for the presence/absence of viruses pathogenic for humans that are distributed in Russia.

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