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EQUINE HERPESVIRUS ABORTION, STILLBIRTH AND NEONATAL DEATH: A BRIEF REVIEW

AVORTUL, MOARTEA FETALĂ ȘI MOARTEA NEONATALĂ ÎN INFECȚIA CU EQUINE HERPESVIRUS: O SCURTĂ RECENZIE

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ABSTRACT | REZUMAT

Equine herpesvirus infection is one of the main problems found in the horse population worldwide. Several types of *Herpesvirus* cause various clinical and pathological conditions, including abortion, stillbirth and neonatal death in horses. Equine herpesvirus type 1 (EHV1) is a member of the *Alphaherpesvirus* subfamily and it is capable of generating several syndromes such as nervous, respiratory, and reproductive in horses. EHV1 is commonly associated with abortion in many countries. In Romania, there are no studies that provide data on the incidence of EHV1-related abortion in horses. The spread of the infection is usually airborne, by direct contact between the individuals, but it can also occur indirectly by personnel and fomites. The state of latency and the capacity of reactivation are the main features of the epidemiology of these infections; thus, those are the key factors of the ubiquitous distribution of EHV-1 and EHV-4 in the horse population. A main aspect of the EHV-1 life cycle that separates it from the one of EHV-4 is that it infects a higher variety of cell types: lymphoid cells, neuronal cells, endothelial cells, and respiratory epithelial cells, while EHV-4 has a tropism mainly for epithelial and neuronal cells, with a limited potential for the infections of endothelial and lymphoid cells. The diagnosis methods of EHV1 infection include anatomopathological examination, immunohistochemistry, molecular and serological analyses. This article reviews the incidence of EHV infections, the strains of EHV associated with abortion, stillborn and neonatal death in horses, focusing on the EHV1, the pathological features and the main techniques used in the diagnosis of equine herpesvirus infection.

Keywords: abortion, diagnosis, equine, herpesvirus infection, pathology

Infecțiile cu Herpesvirus ecvin reprezintă o problemă majoră în cadrul populațiilor de cabaline din întreaga lume. Există numeroase tipuri de herpesvirus ecvin asociate cu diferite stări clinice și patologice, precum avort, fetuși născuți morți și moarte neonatală. Herpesvirusul ecvin de tip 1 (EHV1) face parte din subfamilia *Alphaherpesvirus* și poate reprezenta cauza mai multor sindroame de tip: nervos, respirator și reproducător. EHV1 este deseori asociat cu avortul în numeroase țări, iar in momentul de față, nu există studii care să ofere date despre incidența avorturilor la ecvine, cauzate de EHV1 în România. Răspândirea infecției este realizată, de obicei, pe care aeriană, prin contact direct între indivizi, dar poate să aibă loc și indirect prin intermediul unor obiecte contaminate. Starea de latență și capacitatea de reactivare reprezintă principalele caracteristici în epidemiologia infecțiilor cu acest virus, fiind factorii cheie ai distribuției globale, ubicvitare a EHV1 în rândul populației de cabaline. Un aspect esențial al viremiei cauzate de EHV1 care îl diferențiază de EHV4 este faptul că infectează o varietate mai mare de tipuri celulare, precum celulele limfoide, neuronale, endoteliale și epiteliale respiratorii, în timp ce EHV4 are un tropism orientat spre celulele epiteliale și neuronale și unul limitat pentru infecțiile celulelor endoteliale și limfoide. Metodele de diagnostic în infecția cu EHV 1 includ: examenul anatomopatologic, imunohistochimia, analize moleculare și serologice. Lucrarea își propune să ofere noi date despre incidența infecțiilor cu EHV, tulpinile de EHV asociate cu avort, fetuși născuți morți și moarte neonatală la cabaline, caracteristicile patologice și tehnicile utilizate în diagnosticul infecției cu herpesvirus ecvin.

Cuvinte cheie: avort, diagnostic, ecvine, infecție cu herpesvirus, patologie

Herpesvirus infection represents one of the main problems encountered in the horse population worldwide. There are several types of *Herpesvirus* associated with various clinical and pathological conditions. Thus, the most common herpesvirus infection in horses is produced by EHV type 1, followed by types 5 and 3. In many cases, *Herpesvirus* type 4 infection is identified in the same individuals affected by EHV1. Type 2 EHV infection is usually sporadic, and EHV5 infection is rarely encountered in adult horses and is often associated with EHV2 infection. EHV3 infection is generally difficult to be diagnosed because the disease often occurs

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without clinical signs (34). Mainly, in herds of horses, herpesviruses are responsible for the occurrence of respiratory disorders and abortions (38). In the case of *Herpesvirus* type 1 infection, the main aspect that occurs in horses is interstitial pneumonia in young animals and abortions in the third trimester of pregnancy. In both forms, economic losses are very high (13). In Romania, this disease was diagnosed in 1940 in several groups (51). Because of the illnesses and abortions that it produces, especially in the large agglomerations of horses, it causes massive economic loss. This article reviews the strains of EHV associated with abortion, stillborn and neonatal death in horses, the pathological features, and the main methods used in the diagnosis of equine herpesvirus infection.

ETIOLOGY AND TAXONOMY

Infection with herpesviruses among domestic animals can be the main cause of reproductive failure and abortion. Different subfamilies of *Herpesviridae* are related to abortions in different species: the *Alphaherpesvirinae* is the most involved, and it can be found in all domestic mammals, such as horses, bovine, swine, goats, dogs, and cats. *Betaherpesvirinae* can cause abortions in swine, while the *Gammaherpesvirinae* can be involved in reproductive failure in cattle (43). In *Equidae*, *Herpesviruses* are divided into 9 different species, from which the first 5 can occur in horses: Equid alphaherpesvirus 1, 3, and 4, respectively gammaherpesvirus 2 and 5 (55). EHV-1 is involved in abortion, respiratory diseases and neonatal mortality; EHV-2 may cause respiratory and ocular disturbances, EHV-3 is responsible for the occurrence of coital exanthema, and EHV-4 is the etiologic agent of equine rhinopneumonia, but may also cause other respiratory pathologies and rarely abortions (26). Generally, EHV-1 and EHV-4 infections are responsible for the emergence of equine infectious rhinopneumonia and reproductive insufficiency, EHV-2 infection causes infectious keratoconjunctivitis, EHV-3 infection induces coital exanthema (42), and EHV-5 infection is associated with a progressive multinodular pulmonary fibrosis (EMPF) (27). EHV-6 to EHV-9 are linked to infections in wild equids such as donkeys and zebras (4).

EPIDEMIOLOGY OF EHV1 AND EHV4

EHV-1 and EHV-4 are among the best-known and well-characterized respiratory viruses affecting horse populations. EHV-1 and EHV-4 are alphaherpesviruses (members of the *Alphaherpesvirinae* subfamily) that belong to the *Varicellovirus* genus (39). Each *Alphaherpesvirus* has a strict selection of hosts, normally infecting a single target species (42). The two species are closely related but they are genetically distinct. While serological tests confirmed the sporadic infection with EHV-1 also in captive camelids and cervids, EHV-4 was confirmed only in domestic horses (35). Estimates of the prevalence of EHV-1 infection based on viral detection methods vary widely. In a study carried out in horses from slaughterhouses in Europe, EHV-1 was directly isolated from 60% of the horses when the bronchial lymphocytes were examined, and by PCR, EHV-1 was detected in 88% of this population (26). The prevalence of latent infection with equine herpesvirus type 1 can be influenced by the geographic area, health management practices, and other factors. Similarly, the test technology and, more importantly, the taken tissue will have major effects on the sensitivity of the test. The current approximation of the prevalence of latent infection with EHV-1 suggests a rate that exceeds 60%, which may be more limited by detection technology than the current infection rate. For practical purposes, clinicians should assume that most horses are newly infected with EHV-1. There are no studies that provide data on EHV-1 incidence in Romania.

EHV-1 is an enzootic pathogen of horse populations worldwide, and is considered the most important of the five equine herpesviruses currently known, due to the high emotional impact and a significant cause of economic loss in the horse industry (15, 17).

EHV1 is a virus with double-stranded DNA and pericapsidal sheath, belonging to the genus *Varicellovirus*, subfamily *Alphaherpesviridae*, family *Herpesviridae* (17). The main source of infection with EHV is represented by the individuals with a latent infection. When the horses are kept in close confinement during an outbreak, environmental transmission has the main role in the epidemiology of this virus (42).

EHV can persist in the environment for a short period, estimated at 7 days in almost all conditions (9). The surveys done by serological methods showed the presence of EHV-1 and EHV-4 antibodies in almost all adult horses, while the epidemiological studies done in the stud farms revealed that before or immediately after weaning, so in the first weeks of life, the infection is acquired, from the mothers that are without clinical signs (13). The spread of the infection is usually airborne, by direct contact between the individuals, but it can also occur indirectly by personnel and fomites.

The aerosolized droplets that reach the respiratory tract represent the main route of transmission, but it can also happen by inhalation or ingestion of droplets from surfaces (42).

The state of latency and the capacity of reactivation are the main features of the epidemiology of these infections; thus, those are the key factors of the ubiquitous distribution of EHV-1 and EHV-4 in the horse population. The incidence of equine herpesvirus infection in various European and non-European countries is shown in Table 1.

Table 1

Incidence of equine herpesvirus infection in various countries

Country	Incidence (%)	Method(s)	References
Poland	23.1%	PCR	Matczuk *et al.*, 2018 (28)
New Zealand	80-95%	VN, ELISA, PCR	McFadden *et al.*, 2016 (30)
South Africa	30%	IHC, PCR	Schulman *et al.*, 2015 (40)
Iran	16-22%	PCR	Hafshejani *et al.*, 2015 (20)
Germany	89%	PCR	Damiani *et al.*, 2014 (8)
Poland	25.6%	IF	Bazanow *et al.*, 2014 (3)
Germany + Switzerland	85%	PCR	Walter *et al.*, 2013 (52)
Turkey	80%	HP + PCR	Turan *et al.*, 2012 (50)
Poland	50%	VI + IF	Bazanow *et al.*, 2012 (2)
France	24%	PCR	Pronost *et al.*, 2010 (37)
Uruguay	28%	VI, PCR	Easton *et al.*, 2009 (11)
Colombia	100%	VI, VN, IHC, EM, PCR	Cano *et al.*, 2008 (7)
France	14,49%	PCR	Leon *et al.*, 2008 (24)
USA	5.33%	PCR	Brown *et al.*, 2007 (6)
Lithuania	33.3%	PCR	Liutkeviciene *et al.*, 2007 (25)
Argentina	100%	VI, VN	Galosi *et al.*, 2004 (14)
India	57%	VN	Singh *et al.*, 2004 (41)
Australia	24 %	ELISA, PCR	Foote *et al.*, 2004 (13)
Hungary	14.9%	IHC ISH	Szeredi *et al.*, 2003 (46)
Japan	100%	IHC, ISH, PCR	Kimura *et al.*, 2004 (22)
UK	89%	PCR	Gerst *et al.*, 2003 (18)
Japan	100%	IHC, PCR	Mukaiya *et al.*, 2000 (31)

Legend: VI - virus isolation, VN - virus neutralization, ELISA - enzyme-linked immunosorbent assay, PCR- polymerase chain reaction, IHC- immunohistochemistry, HP – histopathology, IF – immunofluorescence, EM- electron microscopy, ISH – in situ hybridization

PATHOGENESIS

There is a variety of cellular tropism within the *Varicellovirus* genus that goes from predominantly neurotropic viruses to predominantly lymphotropic ones. EHV-1 and EHV-4 are somehow in the middle of this spectrum because, during their life cycle, they are able to be both lymphotropic and neurotropic (42). A main aspect of the EHV-1 life cycle that separates it from the one of EHV-4 is that it infects a higher variety of cell types: lymphoid cells, neuronal cells, endothelial cells, and respiratory epithelial cells (23), while EHV-4 has a tropism mainly for epithelial and neuronal cells, with a limited potential for the infections of endothelial and lymphoid cells (47). Primary infection occurs on the level of the respiratory epithelium through cohabitation with infected animals and inhalation of viruses, or in rare cases, through food or contaminated water (15). Following the initial infection of the upper respiratory tract epithelium, the virus starts to replicate, causing the death of epithelial cells which will result in epithelial erosions; from there, EHV-1 will spread to lamina propria in a short period, infecting endothelial cells, lymphocytes, and monocytes, resulting a high degree of cellular viremia (23). The pathogenesis of abortion due to EHV-1 depends on viremia, which induces endothelial cell infection of the endometrium, leading to vasculitis, thrombosis, microcotyledonous infarction, perivascular cuffing, and transplacental spread of the virus at the sites of vascular lesions (34).

EHV-1 infected peripheral blood mononuclear cells that spread the virus to the endothelial cells. If this occurs in the pregnant uterus or central nervous system, the lesions that develop the affected tissue can cause abortion or myeloencephalopathy (16). Experimental infections have shown that different strains of EHV-1 vary in their abortigenic potential and neuropathogenicity. The Ab4 strain of EHV-1 showed a strong ability to induce abortion, while the other strains induced abortion in only a very few mares, despite its well-documented neuro-virulence (15, 16). The mechanism of abortion in EHV-4 infection is not as clear as for the EHV-1, because the studies have shown that these isolates have a low tropism for endothelial cells (45). Thus, EHV4-related abortions occur also because of vascular lesions (42). These isolates exhibit

the ability to invade the allantochorion, causing ischemia at the level of the placenta and foetus (47).

EHV1 AND EHV4-RELATED SYNDROMES

The clinical syndromes associated with the infection with EHV-1 and EHV-4 are well known. Respiratory, neurological diseases and abortions can be caused by an infection with these viruses. Some highly virulent endotheliotropic isolates of EHV-4 can cause abortion, but it remains the main cause of respiratory disease (42).

Abortion

Abortion in mares infected with EHV-1 usually occurs in the last third, but they can be refractory to abortion if the mare encountered the virus earlier than 120 days of gestation (19). The abortion occurs suddenly, usually without any warning signs (44) apart from pyrexia, and without any respiratory clinical syndrome. Abortions caused by EHV-1 infections generally occur in individual mares, sporadically (42). Usually, the foal is expelled inside the intact amnion, with the mare standing up, and sometimes, the foetus can be expelled within the intact allantochorion. A living foal can, occasionally, be born, but it will die shortly after birth (36). Rarely, episodes of "abortion storms" can occur in stud farms where the hygiene precautions are not scrupulously followed (32).

A successful conceiving shortly after an EHV abortion will appear in most mares and foal normally the following year. EHV abortions that occur in successive years are rare, but the mares can eventually become re-infected, so they will abort again (42).

Neonatal EHV foal syndrome

It is a rare disease associated mainly with EHV-1, but it can be, occasionally a cause of EHV-4 infection (33). The foals are born alive, sick at birth or become ill very shortly after that; thus, it is not evident if they are infected in the uterus, or if they acquire the infection from the dam, right after birth. Affected foals show a rapidly progressive lower respiratory tract syndrome, caused by the main viral pneumonia that will develop respiratory distress, hypoxia, and exitus. The individuals can survive up to 14 days, but they will eventually die because of the respiratory disease and a complex of other complications such as lymphoid depletion and profound leukopenia (42).

DIFFERENTIAL DIAGNOSIS

Abortions in horses can have both infectious and non-infectious aetiologies, but a large number of cases are with an uncertain aetiology. Infectious abortions have the highest proportion, and of these, over 50% are of bacterial origin (*Salmonella spp*., *Streptococcus equi, E. coli., Pseudomonas aeruginosa, Staphylococcus* spp., *Leptospira spp*), 42% of viral origin (*Herpesvirus*, *Arteritivirus*, Equine infectious anemia virus), and a small percentage belongs to parasites (*Neopsora spp*., *Theileria equi*, *Babesia caballi*). In the case of non-infectious abortions, the most common cause is twin pregnancies, followed by foetal malformations, umbilical cord strangulation, and uterine torsions (53). Although the anatomopathological aspects are somewhat conclusive for EHV-1 infection, the differential diagnosis is made for equine influenza. In young animals, respiratory symptoms are similar to those in herpesvirosis, but abortions with specific lesions are characteristic to EHV-1 infection (56).

DIAGNOSIS

The certain diagnosis of herpesvirus infection is made by methods, generally accepted as superior to the anatomopathological examination, such as immunohistochemistry, molecular and serological techniques (1). In case of suspicion of an abortion produced by EHV, the diagnosis of certainty is offered by the PCR technique, the virologic examination and the classic macroscopic and microscopic changes. Lungs, liver, adrenal glands, and lymphatic tissues are usually sources of virus. Serological testing of mares after abortion has an insignificant diagnostic value. The confirmation of the neuropathic strain of EHV-1 is done by real-time PCR, on samples collected from nasal secretions, CSF or nervous tissue. A presumptive diagnosis may be based on clinical signs and examination of cerebrospinal fluid (xanthochromia, albuminocytologic dissociation) (26).

Necropsy and histopathology

Grossly, the lungs fail to collapse, are usually swollen, grey or reddish-brown, dense to rubbery, and the rib imprints are present on their surface. Generally, the tracheal lumen and large bronchi contain a fibrinous exudate and oedema fluid. The trachea-bronchial and mediastinal lymph nodes are enlarged, red-grey because of hyperaemia, oedema, and lymphoid hyperplasia. Occasionally, the pulmonary parenchyma shows multifocal areas of necrosis and a significant amount of yellowish transparent fluid is commonly observed within the pleural cavity. The histopathological exam is the main method for the confirmation of EHV infection in aborted foetuses (54) and neonates. The characteristic changes include acute bronchointerstitial pneumonia with vasculitis, thrombosis, syncytial cells and intranuclear viral inclusion bodies in the respiratory epithelial cells, endothelial cells, and macrophages (42). The liver is another organ involved in equine systemic herpesvirus infection. The gross changes include congestion and presence of numerous, 1-2 mm in dia-

meter, white-yellow, randomly distributed foci of necrosis. Histologically, the hepatic parenchyma is severely altered due to foci of coagulative and lytic necrosis, and mild infiltration with neutrophils, lymphocytes, and macrophages. The adjacent hepatocytes contain small acidophilic intranuclear inclusion bodies surrounded by a clear halo (15) The placenta is diffusely thickened due to hyperaemia and oedema, and shows numerous 2-10 mm in diameter, yellow foci of necrosis, occasionally delimited by a ring of congestion and haemorrhage. Histological examination of the placenta reveals acute inflammatory lesions consisting of multifocal necrosis of the chorioallantoic membrane, associated with hyperaemia, oedema, necrotizing vasculitis and thrombosis, and large numbers of neutrophils, macrophages, and scattered lymphocytes. Acidophilic intranuclear viral inclusions are rarely seen with the placenta (29).

Immunohistochemistry

The immunohistochemistry method can be performed on tissue sections to demonstrate the viral antigen expression in the infected epithelial and endothelial cells and can confirm the cause of vasculitis in foetal tissues and placenta (42). The positive immunoexpression (brown labelled cell nuclei) is usually observed in the pneumocytes, bronchial epithelial cells, hepatocytes, endothelial cells of sinusoids, macrophages, and in the chorionic villi of the placenta (15).

Immunofluorescence

The direct immunofluorescence (IF) test is a rapid and simple way for demonstration of the viral antigens from frozen sections of placental and foetal tissues that have acceptable sensitivity and specificity to be used as the front-line diagnostic test. The test requires a live virus to be present in the sample (42).

Polymerase Chain Reaction

Several PCR tests are approved for the detection of DNA of the equine herpesviruses, with different primes capable of distinguishing the different EHVs (12, 24). In optimum conditions, this test is extremely sensitive and capable of detecting the target viral DNA (5). However, usually the clinical samples are not 100% pure, containing inhibitors of the polymerase enzyme and presenting degradation of the viral DNA (21). In a recent report, the sequence analysis of a portion of the glycoprotein C gene amplified by PCR confirmed EHV-1 activity (11).

Virus isolation

Virus isolation is the "gold standard" test for laboratory diagnosis of EHV infections that gives certain evidence of the presence of the virus in the samples such as blood, placental and foetal tissues (42). This test involves the demonstration of the cytopathic effect in cell cultures inoculated with supernatant from the clinical samples, using RK-13 cells (48).

Serology

Using serological tests, a retrospective diagnosis of EHV-1 and -4 infection can be established that can provide serious information for longitudinal surveillance. After EHVs reach the organism, an initial rapid increase in IgM antibody will occur, detectable after 4 days, with a peak at 20-30 days and returning to the baseline values at 60-80 days (49). A later, but more sustained increase in IgG antibodies will occur, which is detectable at 8-10 days, with a peak at 30-40 days, that will persist for more than 9 months (10).

Haematology

Usually, the practitioners are collecting blood samples from horses with suspicion of EHV infection for hematologic evaluation. This method can provide only a nonspecific mark of a viral infection, so it is not used for the establishment of the diagnosis. Initially, in the first 7-10 days post-infection, a transient leukopenia accompanied with lymphopenia occurs, that will be replaced by a leukocytosis with lymphocytosis until the 21st day after infection. However, this variation in the haematology parameters is difficult to correlate to gain supportive evidence of EHV infection (42).

Cerebrospinal fluid (CSF) analysis

Cerebrospinal fluid collected from an individual with EHM infection may show an increased total protein concentration, without an increase in the count of nucleated cells. The CSF can also present a yellow discoloration, as a sign of xanthochromia, which is caused by a high level of red blood cell breakdowns and increased protein concentration. These changes in the CSF, correlated with the characteristic clinical sign can be suggestive, but they cannot be used to diagnose EHV infection (42).

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