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Clinical impact, diagnosis and control of Equine Herpesvirus-1 infection in Europe

European Food Safety Authority (EFSA),
Andrea Carvelli, Søren Saxmose Nielsen, Romain Paillot, Alessandro Broglia and Lisa Kohnle

Abstract

Equine herpesvirus-1 (EHV-1) can affect the entire equine sector in EU, and the large outbreak reported in 2021 in Spain drew attention to the needs of the European Commission for scientific advice for the assessment of EHV-1 infection within the framework of Animal Health Law. EHV-1 is considered endemic in the EU; its main risk is linked to the characteristic of producing latent life-long infections. These can reactivate producing clinical disease, which can include respiratory, abortive and possibly fatal neurological forms. From the epidemiological and genomic viewpoint, there are no specific neuropathogenic EHV-1 strains; the respiratory, reproductive and neurological signs are not found to be strain-specific. This was also the case of the virus that caused the outbreak in Valencia (Spain) in 2021, which was genetically closely related to other viruses circulating before in Europe, and did not present the so-called neuropathogenic genotype. The outbreak reported in Valencia was followed by wide geographic spread of the virus possibly due to a delay in diagnosis and late application of biosecurity measures. The recommended and most sensitive diagnostic test for detecting EHV-1 is PCR performed on swabs collected according to the type of clinical signs. Serological assays on paired blood samples can help to detect a recent infection, while no diagnostic methods are available to detect EHV-1 latent infections. Safe movements of horses can be ensured at premovement phase by testing and issuing health certificates, and by isolating animals upon arrival at new premises with regular health monitoring. In case of suspicion, movements should be forbidden and EHV-1 infection early detected/confirmed by validated diagnostic tools. During outbreaks, no movements should be allowed until 21 days after the detection of the last case. In general, vaccination against EHV-1 should be promoted, although this offers limited protection against the neurological form of the disease.

Keywords: Equine herpesvirus-1 infection, horse, Equidae, latent infection, PCR, diagnosis, risk mitigation

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Correspondence: biohaw@efsa.europa.eu

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Summary

The European Commission needed scientific advice for the assessment of EHV-1 infection within the framework of Regulation (EU) 2016/429 ('Animal Health Law' (AHL)) and related Commission Delegated Regulation (EU) 2018/1629 and Commission Implementing Regulation (EU) 2018/1882. The infection with EHV-1 is not included in the list of diseases in the AHL, but an outbreak of EHV-1 in the context of an equestrian competition in 2021 has drawn the attention to this infection which can affect high value breeding and sport horses and the equine sector as a whole.

In January 2022, EFSA published an assessment on EHV-1 infection according to the criteria provided for in Article 7 and 8 and Annex IV of the AHL.

The same mandate from the European Commission further requested to (i) assess the potential of EHV-1, notably its neurotropic variant, to affect equine animals in the Union; (ii) assess the performance of the available diagnostic methods for the detection of EHV-1, including its neurotropic single point mutations; and (iii) describe and assess possible methods and feasible risk mitigation measures to ensure safe international and EU trade in equine animals and their germinal products, including movement restrictions. These are reported here.

The potential of EHV-1, which is widely present and considered endemic in the EU, to affect horses relies on its characteristic to produce a latent lifelong infection in the host. The latent virus can reactivate leading to clinical disease. Such clinical manifestations are rare events and can include respiratory signs, abortions and neurological signs, the latter with possible fatal outcomes. All these clinical forms depend on several factors linked to host characteristics, management/environment, although there is still a large uncertainty about the causality of neurological form. Regarding the so-called neurotropic variants, the epidemiological and genomic evidence suggests that there are no specific neuropathogenic EHV-1 virus strains, and no consistent associations were found between strains and clinical forms to date. This was also the case of the virus that caused outbreak in Valencia in 2021, which was genetically closely related to other viruses circulating before in Europe, and did not present the so-called neuropathogenic genotype. The outbreak reported in Valencia was followed by wide geographic spread of the virus possibly due to a delay in diagnosis and late application of biosecurity measures.

Considering the performance of the diagnostic methods, the recommended and most sensitive test for the direct detection of EHV-1 infection in the presence of respiratory signs is PCR performed on nasopharyngeal swabs. Genotyping or phylogeny (e.g. MLST, whole genome sequencing) should be used as an adjunct to epidemiological investigations. In case of abortion or neurological signs, the related sample matrices should be taken for diagnosis.

Paired blood samples at least 2-week interval can be used to detect a recent infection with serological assays. A seroconversion is confirmed with a fourfold or greater rise in virus-specific antibody titre by either virus neutralisation (VN) assay or complement fixation (CF) test. Up to date EHV-1 latent infections cannot be detected by any diagnostic method.

Safe movement/trade of horses can be ensured by risk mitigation measures about prevention but also during outbreaks. Among the former, the measures to be applied before movement of horses to new premises include premovement testing and issue of health certificate, proving that animals do not show clinical signs, its vaccination status and that no cases were detected in the premise of origin in the previous 21 days. Upon arrival to a new premise, horses should be isolated, with regular monitoring of health conditions and rectal body temperature. In general, vaccination against EHV-1 should be promoted, although this offers limited protection against the neurological form of the disease.

During outbreaks and/or in case of suspicion of infection, the application of movement restrictions and early detection of EHV-1 infection by validated diagnostic tools is of key importance. No movements should be allowed during the period of the outbreak as long as new cases are detected, and for 21 days after the detection of the last case.

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1. Introduction

1.1. Background as provided by the European Commission

Chapter 2 of Part 1 of Regulation (EU) 2016/429 ('Animal Health Law' (AHL)) provides for the criteria for listing of diseases, listing of species and categorisation of listed diseases. Using those criteria, EFSA has provided valuable assistance to the Commission in preparation of tertiary legislation under Regulation (EU) 2016/429 on the list of diseases as well as the categorisation, that lead to the adoption of Commission Delegated Regulation (EU) 2018/1629 and Commission Implementing Regulation (EU) 2018/1882. The outbreak of equine herpesvirus-1 (EHV-1) in the context of a major equestrian competition in early 2021 has drawn the attention to this infection. As infection with EHV-1 is not included in the list of diseases in Annex II to the AHL, but affects high value breeding and sport horses and the equine sector as a whole, the Commission may be asked to place infection with EHV-1 onto that list. An assessment of EHV-1 with a view to its possible inclusion into the list of diseases would be necessary in accordance with a set of criteria provided for in the AHL.

Hence the Commission needs scientific advice for the assessment of the significance of infection with EHV-1 within the framework of this already known listing and categorisation according to the AHL (https://doi.org/10.2903/j.efsa.2017.4783), in the same manner it was carried out previously (https://doi.org/10.2903/j.efsa.2017.4946).

The criteria provided for in Article 7 and 8 and Annex IV of the AHL shall be used as a basis for this analytical assessment. The risk manager needs a scientific advice in order to:

1) assess if EHV-1 infection causes disease for which control measures at the EU level are justified;
2) proceed with the profiling of the disease in view to its categorisation; and
3) assign listed species to EHV-1 infection identified as eligible for EU intervention.

The Commission has identified the following issues for which concrete elements of science may provide good basis for formulating policies and/or adapt current approach:

- provisions for safe trade (entry into the Union and trade within the Union) in equine animals and their germinal products from countries affected by EHV-1;
- identifying possible routes and risks of spreading EHV-1 between equine animals resident in the Union and those imported from third countries;
- effects of the respective infection of equine animals with EHV-1, including aspects stemming from different susceptibility of various species to EHV-1 and of different virulence of various EHV-1 viruses;
- measures to monitor occurrence of EHV-1 in equine animals and mitigate mortality due to EHV-1 infection, whether regulatory measures or non-regulatory ones.

1.2. Terms of Reference as provided by the European Commission

1.2.1. Scientific opinion in accordance with Article 29 of Regulation (EC) No 178/2002

In accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to provide a scientific opinion as regards the listing and categorisation under the AHL of EHV-1, including the following:

1) assess, following the criteria laid down in Article 7 of the AHL, its eligibility of being listed for Union intervention as laid down in Article 5(3) of the AHL;

if found eligible to be listed for Union intervention, provide:

2) an assessment of its compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;
3) a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.

This listing and categorisation should be executed according to the method defined in Scientific Opinion 'Ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law' (EFSA AHAW Panel, 2022a).

1.2.2. Scientific and technical assistance in accordance with Article 31 of Regulation (EC) No 178/2002[1]

Where the outcome of the assessment in point I suggests listing of the disease in accordance with Article 5(3) of Regulation (EU) 2016/429, the Commission asks EFSA to provide, in accordance with Article 31 of Regulation (EC) No 178/2002, scientific and technical assistance concerning the following:

a) assess the potential of EHV-1, notably its neurotropic variant, to affect equine animals in the Union;
b) assess the performance of the available diagnostic methods for the detection of EHV-1, including its neurotropic single point mutations;
c) describe and assess possible methods and feasible risk mitigation measures to ensure safe international and EU trade in equine animals and their germinal products, including movement restrictions.

1.3. Interpretation of the Terms of Reference

The present document contains an assessment according to the ToRs points a, b and c as in Section 1.2.2.

2. Data and methodologies

The assessment presented in this report is based on the evidence previously collected in the scientific opinion from EFSA (EFSA AHAW Panel, 2022b), literature and on expert opinion.

3. Assessment

At least nine herpesviruses infect equids, but EHV-1 poses the most serious health risks to the horses worldwide. The disease caused by EHV-1 or the closely related EHV-4 in horses is usually called Equine Rhinopneumonitis (ER). EHV-1 and EHV-4 share genetic, antigenic and epidemiological characteristics, but they significantly differ in pathogenicity. EHV-4 causes less severe disease, while EHV-1 infection can lead to clinical forms varying from subclinical infection to severe disease involving the respiratory system, abortion and neurological disease (the latter called Equine Herpesvirus Myeloencephalopathy (EHM)) with a possible fatal outcome (OIE, 2019). An extensive assessment of the disease caused by EHV-1 was presented in an EFSA output addressing the first part of the mandate from European Commission (EFSA AHAW Panel, 2022b).

The infection with EHV-1 is listed by the OIE, while it is not subject to compulsory notification in the European Union; therefore, EHV-1 infection is often under-reported despite the wide presence in Europe and most countries globally.

3.1. Potential of EHV-1 to affect equine animals in European Union

The EHV-1 infection is widely present and is considered endemic in the EU (EFSA AHAW Panel, 2022b). The potential of EHV-1 to affect horses, as other herpesviruses, relies on the fact that, once infected, the host remains lifelong infected. The virus can then remain latent or it can reactivate leading to clinical disease. Therefore, a clinical form of EHV-1 infection can be the result of a new infection in a susceptible, naïve horse; or a reactivation of a latent infection, which is the most common source of EHV-1 infection; or a new infection in a previously infected horse. The latter is possible because the post-infection immunity lasts up to about 6 months (Reed and Toribio, 2004; Slater, 2014; OIE, 2019). Details on the latency and duration of infection are reported in EFSA AHAW Panel (2022b).

Despite that the EHV-1 infection is considered to occur with a high (> 60%) prevalence (Allen, 2002a; Lunn et al., 2009) in equine populations worldwide (Allen, 2002b; Lunn et al., 2009; Dunowska, 2014; Slater, 2014; Oladunni et al., 2019; OIE, 2019), the majority of the EHV-1-infected horses do not show any clinical signs, because most infections are latent. If clinical disease occurs, the severity of the clinical form depends on factors such as age, immune and health status, the housing conditions (Nugent and Paillot, 2009; Kydd et al., 2012; Slater, 2014; Oladunni et al., 2019; Zarski et al., 2021). EHV-1 can cause three different syndromes, i.e. respiratory disease, abortion and neurological disease (EHM). The respiratory disease presents a wide range of morbidity, the abortion

[1] These ToRs will be addressed in a separate scientific report by March 2022.

can be sporadic or epidemic (the so-called 'abortion storm') and may depend on animal density and biosecurity measures. The EHM is a rare event, although the presentation and the severity of clinical signs are highly variable. The mortality is usually low, ranging from 0.5% to 10% reported in published studies regarding affected premise (EFSA AHAW Panel, 2022b).

The extent of an outbreak depends on numerous factors, of which some are host related (like age, sex, immune status, concurrent disease, stress level), and others are at country or holding level specific (e.g. animal density, number and conditions of horse movements, biosecurity and control measures applied), while the presence of official notification of EHV-1, EHV-1 case definition, horse industry management may also affect the reported outbreak characteristics (Goehring et al., 2006; Allen, 2008; Perkins et al., 2009; Traub-Dagartz et al., 2013; van Galen et al., 2015; Pusterla et al., 2016; Garvey et al., 2019; Dunuwille et al., 2020; Pusterla et al., 2020).

Neurological form and neurotropic virus variants

The EHM is a rare event; it can be the sequelae of the EHV-1 respiratory form, but can occur also without previous respiratory signs (Slater, 2014). The presentation and the severity of neurological signs are highly variable (Dunowska, 2014). EHM can be observed in outbreaks of different extent (Henninger et al., 2007; USDA, 2011; Barbić et al., 2012; Burgess et al., 2012; Sutton et al., 2021).

The proportion of horses showing neurological signs on premises, where EHM outbreak events were reported, has been observed to 1% and 15% in Croatia, France, Canada (Barbić et al., 2012; Weese, 2017; Sutton et al., 2020), and 6% and 34% in the USA (Henninger et al., 2007; USDA, 2011) in longitudinal or retrospective studies carried out in EHV-1 outbreaks. In the Netherlands, six EHM outbreaks were investigated and the proportion of horses with neurologic dysfunction out of present horses in the premises ranged from 10% to 33% (Goehring et al., 2006).

In the past, the ability of the virus to cause EHM was thought to be associated with specific EHV-1 strains, the so-called neuropathogenic strains, which presented a specific DNA polymerase gene mutation (gene open reading frame 30: G2254/N752). Nevertheless, virus strains without this mutation have been isolated in several outbreaks with horses suffering with neurological signs (Goehring et al., 2006; Nugent and Paillot, 2009; van Galen et al., 2015; Garvey et al., 2019; Dunuwille et al., 2020; Pusterla et al., 2020; Sutton et al., 2020; Vereecke et al., 2021). For example, the virus causing the most severe EHV-1 epidemic outbreak in the last decades in Europe with several cases of neurological disease, like the one reported in Valencia at the beginning of 2021, did not belonged to the so-called neuropathogenic strains and presents the A2254/D752 mutation, as described by Vereecke et al. and Sutton et al. (2021). This outbreak originated in an International Horse Jumping event hold in Valencia (Spain), where 850 horses were gathered. A total of 114 horses showed clinical signs or tested positive using PCR.[2] Due to horse movements from the event venue to their usual holdings, the disease rapidly spreads to over 30 premises in different countries, including Belgium, Denmark, Spain, France, Germany, Italy, Qatar, Slovakia, Sweden and Switzerland. The site was locked down with 160 horses from 15 different countries staying on site, and 118 were infected with EHV-1. This led Federation Equestre Internationale (FEI) and competent bodies to cancel sport horse events in 12 European countries. At least, 20 affected horses were euthanised (Kubacki et al., 2021; Termine et al., 2021; Vereecke et al., 2021). The magnitude of this event caused an increasing awareness and concern about EHV-1 infection in the European Union.

Nasopharyngeal swab samples were collected from 67 of the horses that remained in the Valencia facility and tested for genomic analysis (Sutton et al., 2021). Nineteen PCR-positive samples were tested by the allelic-discrimination analysis for a single-nucleotide polymorphism (SNP), which was the A2254G, within open reading frame (ORF) 30. All the strains were found to be A2254, so-called non-neuropathogenic strain. Two strains isolated from two horses were also characterised by multilocus sequence typing (MLST). This showed that the virus associated with the outbreak of EHM in Valencia was closely related to other viruses circulating for several years in Europe (Sutton et al., 2021). Therefore, up to date, the epidemiological and genomic evidence suggests that there are no specific neuropathogenic EHV-1 virus strains, and that any strain can lead to respiratory, reproductive and neurological signs.

Further analysis of the sequences of the different ORF fragments used for the MLST revealed a mutation of the two strains isolated in Valencia. This mutation was not identified in sequences obtained in GenBank from strains isolated previously in the United Kingdom, United States, China,

[2] https://www.rfhe.com/wp-content/uploads/2021/03/Informe-foco-rinoneumonia-MAPA-17-03-21.pdf

Australia, Belgium, New Zealand, Japan or India. Therefore, this polymorphism could represent a possible marker for the virus strain linked to the Valencia outbreak, and it may be useful to investigate the extent of the spread of the virus strain in different countries. This tracking tool would be particular important considering that the Federation Equestre Internationale (FEI) and competent bodies and authorities cancelled sport horse events in 12 European countries after the outbreak being notified (Sutton et al., 2021).

The outbreak was followed by wide geographic spread of the virus possibly linked to a delay in diagnosis and late application of biosecurity measures. The epidemiological pattern observed during this epidemic was similar to other outbreaks reported in last decades in Europe (Goehring et al., 2006; Gryspeerdt et al., 2011; Pronost et al., 2012; Walter et al., 2013; van Galen et al., 2015; Sutton et al., 2020) and worldwide (Henninger et al., 2007; Tsujimura et al., 2011; Burgess et al., 2012; McFadden et al., 2016; Negussie et al., 2017; Pusterla et al., 2020), with movements of horses playing a relevant/ important role in the spread of the virus to other premises.

3.2. Performance of the available diagnostic methods for the detection of EHV-1

Numerous diagnostic methods and tools are available and used to confirm cases of EHV-1 or EHV-4 infection (e.g. PCR, virus isolation, measures of seroconversion). However, the main limitation for disease control strategies is that it is not possible to detect latent infections and to serologically differentiate infected from vaccinated animals (DIVA). The diagnostic methods are described in detail in EFSA AHAW Panel (2022b). In this section, the aspects related to the choice of a diagnostic test for clinical forms, latent infection and genetic mutations of EHV-1 are discussed.

3.2.1. Diagnosis of clinical cases

As indicated in the OIE Terrestrial Manual, the recommended method for detection and identification of EHV-1 (and the closely related EHV-4) in animals with clinical signs and during outbreaks is the PCR (direct detection of the virus nucleic acids). PCR results could be supported by virus isolation in cell culture.

The best sample matrices for PCR diagnosis are nasopharyngeal swabs in case of respiratory signs, blood, tissue samples from aborted fetus in case of abortion and cerebrospinal fluid or post-mortem brain and spinal cord samples in case of neurological signs (Lunn et al., 2009).

Up to date PCR is considered to be the most sensitive and specific test for the detection of EHV-1 in nasal and nasopharyngeal swabs, although an accurate quantification of the test performance is not possible because the available publications are outdated and provide non-comparable information about test sensitivity and specificity (Elia et al., 2006; Hussey et al., 2006; Perkins et al., 2008). On the other hand, since the development of these early tests, quality procedures have been published more recently, including inter-laboratory assays, to ensure test performance (e.g. in France: Afnor Norm U47–600, 2015). Several laboratories have ISO17025 accredited qPCR tests for EHV1 and proficiency panels of clinical samples are distributed internationally by the OIE Reference Laboratory.

Nasopharyngeal swab is the most suitable sample to detect virus shedding in nasal secretion, and it must be preferred to nasal swab in which the amount and duration of virus shedding detection could be reduced (Paillot et al., 2013). However, no controlled trials are available to compare the two matrices and more details on the sensitivity given different length of swab sticks would be needed. A field study conducted during an EHV-1 outbreak in the USA has reported that nasal swabs are a viable alternative when compared to the less tolerated nasopharyngeal swabs (Pusterla et al., 2008). Nasopharyngeal lavage is another possible sample matrix although it has no advantage when compared to nasal/nasopharyngeal swabs, and is less tolerated by horses. As such, lavage is not frequently used in the field if there is a suspicion of EHV-1 infection.

The timing of collection of the sample after EHV-1 infection is also very important for the accurate diagnosis. EHV-1 shedding in the respiratory tract could be detected by qPCR from 1 day after infection to 2–3 weeks, depending on the level of infection and the immunological status at the time of infection. Cell-associated viraemia could be detected in blood around 5 days after infection, up to 2–3 weeks depending on the level of infection and immunological status.

Inconclusive PCR results could be confirmed by seroconversion in particular by using paired blood samples, taken at least 2 weeks apart (e.g. acute and convalescent samples) in order to document seroconversion and as post-event confirmation. A fourfold or greater rise in virus-specific antibody titre

detected by either virus neutralisation (VN) assay or complement fixation (CF) test (the two serological tests generally used is indicative of recent infection (EFSA AHAW Panel, 2022b). The ELISA assay (e.g. SVANOVIR[3]) is available and occasionally used. This approach can be used in outbreak situation to confirm or exclude recent virus circulation. Nevertheless, this could be complicated by the endemic nature of EHV-1 and EHV-4 and their cross-reactivity.

Point-of-care tests for the detection of EHV-1/4 nucleic acid in respiratory secretion are currently being developed or commercialised (e.g. Enalees Epona test in France). Such tests have clear advantages in terms of faster detection and faster implementation of control measures and treatment, although their performance (sensitivity and specificity) and their potential impact on field surveillance (e.g. reduced reporting, reduced sample availability for subsequent virus strain characterisation and isolation) remain to be evaluated.

For the purpose of safe movement of horses, an important point is detection of those animals that may be infectious but not showing clinical signs. Furthermore, some animals can be PCR positive due to residual viral particles in the nasopharynx although they do not shed viable and infectious virus. More complicating, there are also animals that can test intermittently positive. During epidemics, there may be infected animals that show low level of virus shedding or viral load. Still, PCR test on nasopharyngeal swabs is the best option to detect shedder animals.

3.2.2. Diagnosis of latent infection

To date, there is no diagnostic test available to detect latent infections, and this is one of the main limitations for the control of the spread of EHV-1 infection.

3.2.3. Characterisation of single point mutations

EHV virus strain characterisation and typing assays have improved in recent years. They could be used to provide epidemiological links, but this still remains infrequent in the field and their results may present difficulties in the interpretation in the field.

Single-nucleotide polymorphisms (SNP) may be helpful for epidemiological purposes (i.e. tracking the infection source and spread) and some of them were also initially thought to be pathological markers, such as the SNP identified at position 2,254 in the DNA polymerase gene (ORF 30) that was thought to be correlated with neurological disease; therefore, it was considered as a neuropathic marker (Nugent et al., 2006). On the basis of this point mutation, the majority of diagnostic laboratories in the USA rely solely on allelic discrimination qPCR assays to detect EHV-1 (Pusterla et al., 2021).

To date, the importance of this neuropathic marker is arguable (EFSA AHAW Panel, 2022b). While EHV-1 strains carrying the A2254 mutation are frequently (but not exclusively) isolated from abortion cases, EHV-1 strains isolated from neurological disease cases are carrying mutation either. Recently, a third 2254 mutation (C2254) was isolated from a large outbreak in France (Sutton et al., 2020), and subsequently reported in the USA (Pusterla et al., 2021), which raised further questions on the prognostic importance of this marker (Sutton et al., 2020). Given this evidence, unfortunately, the molecular diagnostic approach based only on allelic discrimination, may lead to missed EHV-1 cases as exemplified by the outbreak reported in USA (Pusterla et al., 2021) and by the recently reported outbreak from France (Sutton et al., 2020). It is, therefore, recommended to use molecular laboratories that either incorporate a universal EHV-1 target gene or expand their ORF 30 SNP assays to include the new genotypes. Close clinical monitoring of at-risk horses remains the main strategy in disease prevention.

Another specific SNP marker present in the EHV-1 strains involved in the 2021 EHV-1 outbreak in Valencia (Spain) was recently identified and used to trace specifically infected horses (Sutton et al., 2021).

For phylogenetic studies, other genomic tools are available, such as whole ORF 30 sequencing, whole genome sequencing and MLST, details are provided in EFSA AHAW Panel (2022b). For the purpose of detecting concentrations of live infectious EHV-1, cell-culture isolation of EHV-1 is used. Expert laboratories should ensure the validity of the tools over time, i.e. monitor the appearance of mutations in primer and probe targets that could alter the screening tool.

[3] https://www.svanova.com/products/equine/ep01.html

3.3. Risk mitigation measures against EHV-1 to ensure safe international trade in equine animals and their germinal products

The risk mitigation measures against EHV-1 infection are presented and assessed in details in EFSA AHAW Panel (2022b). In particular, the measures to ensure safe trade and movement of horses, since this is the main driver for disease spread between premises, include early detection of EHV-1 infection by well-established and validated diagnostic tools (discussed above) and consequent application of biosecurity measures and movement restrictions, as the most effective approach.

The biosecurity measures for safe trade/movement include mainly but not limited to:

- A health certificate stating at least that no clinical signs are shown by the animal, its vaccination status and that no cases have been reported in the premise in the previous 21 days. The certificate should not be older than 48 h before movement to a new premise or herd.
- Premovement testing, which may be useful in some circumstances, such as in case of suspicion in the area, in case of alert in the country or region. Since EHV-1 infection is not notifiable, the alert would be based on indications by health authorities or equestrian/sport/race/breeders' organisations, which may ask for premovement tests.
- Isolation on arrival of horses in a new premise, with regular health monitoring, in particular rectal body temperature monitoring and appropriate testing if required.
- Disinfection of premises and transport vehicles.
- Segregation of visiting horses from resident horse populations, especially segregation of pregnant mares, which should be all vaccinated.
- Reduced stress, in general and in particular during transport. Stress associated with weaning, commingling, poor health (e.g. parasitism) and transportation are recognised as known risk factors for the reactivation of latent EHV-1 and EHV-4 infection.
- Vaccination (details in EFSA AHAW Panel, 2022b) is useful to prevent abortion and respiratory disease although the evidence of its efficacy against neurological disease is limited, due to the lack of an appropriate EHM model. In any case, vaccination should be encouraged for horses with frequent movements to decrease the insurgence of respiratory signs, although, since there is no evidence that vaccine can protect against the neurological form, vaccination alone cannot guarantee safe movement.

For different categories of horses and situations, mitigation measures to ensure safe movement and trade may also foresee as follows.

- Competition horses as well as any type of gatherings of animals, such as fairs, public sales, etc.: Daily fever monitoring during events can be important for early detection. Those suspected animals should be then confirmed by laboratory test. For the latter, a readily available laboratory with sufficient capacity to process samples quickly with recognised methods is needed. During sport events, isolation facilities would be also necessary, and the best would be to organise such events in off-site facilities. When indoor gathering of horses coming from different premises and/or geographical areas is unavoidable, the location should be well ventilated, given the epidemiological characteristics of EHV-1, to be transmitted mainly by direct contact and respiratory droplets.
- Breeding horses and breeding centres: The vaccination of mares is of utmost importance, to protect from abortion. The vaccination status and the booster schedule should be checked. Awareness about the importance of this as well as the need of a health certificate should be raised with mares' owners. Foaling mares should have the least contact with other horses, they should be isolated in case of outbreaks, a visit schedule should be implemented to limit mares/horses interaction on site, as well as the flow of animals on the premise should be organised to avoid unnecessary interaction. Hygiene measures and disinfection for the personal and equipment used, as well as dedicated equipment for each mare, should be used, if possible.
- For horses kept for meat production, similar considerations as for live animals are valid, since their import movements can be followed by fattening periods for weeks or months, and they are not slaughtered immediately. In case of outbreak, standstill is an important measure and should be applied.

About role of trade with germinal products, in particular semen as possible vehicle of transmission of EHV-1, there is not much neither clear evidence available. Hodder et al. (2007) analysed semen samples

taken from 50 stallions for the presence of EHV-1 DNA using real-time Taqman PCR assay, and DNA was not detected in any of the samples analysed, while in the study carried out by Hebia-Fellah et al. (2009) EHV-1 DNA was detected in the semen of 51 of the 390 stallions sampled (13%). These results should be carefully interpreted, because the presence of virus nucleic acids can be detected in different matrices, without being necessarily relevant in epidemiological terms. In fact, there has never been epidemiological evidence of semen as possible source of EHV-1 infection in equine industry, despite its large use in artificial insemination, nor evidence of infectivity of semen demonstrated in cell culture. Up to date, there is no evidence of the role of semen as possible vehicle of EHV-1.

Regarding movement restrictions, these can be applied only to animals that are recognised as virus shedders and not to latently infected animals that cannot be identified and are presumably not contagious. Movement bans are particularly effective in case of outbreak, when no movements should be allowed during the period of the outbreak as long as new cases are detected, and for 21 days after the detection of the last case, thus to avoid the infection of new susceptible animals.

The spread of EHV-1 between premises is unusual, still it may happen if infected horses are moved before the infection is diagnosed. This was the case of dispersal of infected horses from an equestrian event such as the Valencia venue in 2021 that resulted in wide geographic disease spread. In this situation, the health check and monitoring of visiting horses (e.g. daily rectal temperature monitoring) and early detection are key in preventing large disease spread.

In case of competition horses, the effectiveness of movement restrictions to contain disease spread is challenged by the frequent movements and short-term residence of horses in these events and conditioned by the early detection of the infected animals. In case of an outbreak detected during an equestrian or race events, like the one in Valencia, movement restrictions must be applied also if hardly feasible and horses must be kept on the affected premise until complete resolution of cases.

4. Conclusions and recommendations

TOR1: To assess the potential of EHV-1, notably its neurotropic variant, to affect equine animals in the Union

- The EHV-1 infection is widely present and is considered endemic in the EU.
- The potential of EHV-1 to affect horses relies on its characteristic to produce a latent lifelong infection in the host. The latent virus can reactivate leading to clinical disease.
- Clinical manifestations are rare and can include respiratory signs, abortions and neurological signs, the latter with possible fatal outcomes.
- The disease is multifactorial and its severity is thought to depend on many factors such as age, housing, immune and health status. Occurrence of neurological form is thought to depend on several factors linked to host characteristics, management/environment, although there is still a large uncertainty about the causality of neurological form.
- The virus that caused outbreak in Valencia in 2021 was genetically closely related to other viruses circulating before in Europe, and did not present the so-called neuropathogenic genotype. The epidemiological pattern observed during this epidemic was similar to other outbreaks reported in last decades in Europe and worldwide.
- The outbreak reported in Valencia was followed by wide geographic spread of the virus possibly due to a delay in diagnosis and late application of biosecurity measures.
- The epidemiological and genomic evidence suggests that there are no specific neuropathogenic EHV-1 virus strains, and that any strain can lead to respiratory, reproductive and neurological signs.

TOR2: assess the performance of the available diagnostic methods for the detection of EHV-1, including its neurotropic single point mutations

- The recommended method for the direct detection of EHV-1 infection in the presence of respiratory signs is PCR performed on nasopharyngeal swabs. This procedure is the most sensitive method that can detect virus from shedding horses. Genotyping or phylogeny (e.g. MLST, whole genome sequencing) should be used as an adjunct to epidemiological investigations.
- In case of abortion or EHM, other sample matrices should be taken for diagnosis: tissue samples from aborted fetus or placenta; cerebrospinal fluid (CSF), post-mortem brain and spinal cord samples, respectively.

- A recent infection can be detected with serological assays in blood serum samples, by taking paired samples at least 2-week interval. A seroconversion is confirmed with a fourfold or greater rise in virus-specific antibody titre by either virus neutralisation (VN) assay or complement fixation (CF) test.
- To date, there is no diagnostic method available to detect EHV-1 latent infections.

TOR3: To describe and assess possible methods and feasible risk mitigation measures to ensure safe international and EU trade in equine animals and their germinal products, including movement restrictions.

The following risk mitigation measures against EHV-1 are key to ensure safe movement/trade of horses and germinal products:

- Health certificate to be issued no more than 48 h before movement to a new premise, stating at least that animals do not show clinical signs, vaccination status and in the previous 21 days, no cases were detected in the premise of origin.
- Premovement testing depending on the epidemiological situation.
- Promoting vaccination against EHV-1 and information on its limitations to protect against neurological disease.
- Isolation of horses on arrival in a new premise, with regular health monitoring, in particular rectal body temperature monitoring.
- Early detection of EHV-1 infection by well-established and validated diagnostic tools and laboratories.
- Application of biosecurity measures, among those cleaning and disinfection of fomites, premises and transport vehicles, as recognised important environmental source of the virus.
- Application of movement restrictions during outbreaks and/or in case of suspicion of infection. No movements should be allowed during the period of the outbreak as long as new cases are detected, and for 21 days after the detection of the last case.
- To date, there is no evidence of the role of semen as possible vehicle of EHV-1.

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Abbreviations

AHL	Animal Health Law
CF	complement fixation
DIVA	differentiate infected from vaccinated animals
EHM	Equine Herpesvirus Myeloencephalopathy
EHV-1	Equine herpesvirus-1
ER	Equine Rhinopneumonitis
FEI	Federation Equestre Internationale
MLST	multilocus sequence typing
ORF	open reading frame

PCR	Polymerase chain reaction
SNP	single-nucleotide polymorphism
ToR	Terms of Reference
VN	virus neutralisation assay