Equine viral rhinopneumonitis

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Summary: Infection of horses by equine herpesvirus 1 (EHV-1) may produce equine rhinopneumonitis, abortion or neurological disease. Two antigenically related subtypes of the virus are distributed world-wide. Apparent differences in characteristics of virulence of the subtype viruses are evident by the association of subtype 1 (S-1) with most cases of abortion and neurological disease and the association of S-2 mainly with respiratory disease in young horses.

The viruses survive as a result of the production of latent infections and their ability to productively reinfect immunologically experienced horses as well as immunologically naïve new additions to herds.

Experiences with the Australian and Japanese population of purebred horses suggest that severe economic losses may occur as the result of introduction of the S-1 virus into populations in which the S-2 virus has been the predominant or sole existing strain.

Because no practically applicable method exists for identification of latently infected animals, there is no reliable basis upon which efficient quarantine regulations may be promulgated. Minimum quarantine of horses to be added to herds should be enforced for no less than 21 days on the destination premises. Pregnant broodmares should be sequestered from other pregnant females until they have foaled. The most efficient prophylactic measures for control of abortigenic disease include institution of management practices which produce the least possible stress along with the use of a planned programme of vaccination.


INTRODUCTION

Equine viral rhinopneumonitis is a result of infection of Equidae by equine herpesvirus 1 (EHV-1). The disease was described first by investigators in our laboratories fifty years ago as a cause of abortion (12). It is now also known to be a cause of respiratory disease (16) and of a pathologically unique encephalomyelopathy (25, 29, 39).

Horses usually become infected by EHV-1 during their first year of life. Their initial infection results in acute, febrile respiratory disease (16). The viral infection produces necrosis of lymphoreticular tissues and vesiculation and desquamation of the respiratory mucosa. The disease presents with fever, inappetence, temperatures...
of 39.5 to 41.5°C, neutropenia, pharyngitis, and tracheobronchitis. It is frequently complicated by bacterial infection. The morbidity rate in groups of immunologically naïve weanling foals is usually 100 per cent; the mortality rate is negligible. When death occurs it is most commonly a result of viral pneumonia complicated by bacterial infection.

Pregnant mares infected by EHV-1 may abort. Abortion usually occurs in the terminal three months of pregnancy (21). Although abortion induced by experimental inoculation may occur earlier, naturally occurring abortion caused by this virus infection has not been observed prior to the fifth month of gestation; abortions during the sixth and seventh month occur rarely. Most abortions occur within a period of three weeks after mares become infected but prolonged incubation periods between infection and abortion have been observed (17).

Infection of horses of any age by EHV-1 may result in occurrence of a disseminated vasculitis (Bryans, unpublished observations; 25). The primary lesion of this disease appears to be the result of an Arthus reaction induced by viral antigen-antibody complexes in endothelial cells (25, 34, 46). Endothelial damage and necrosis of vessel walls leads to thrombosis which produces ischemic damage. When such lesions occur in sufficient numbers or functionally critical sites of the central nervous system, they become evident by development of signs of damage to both sensory and motor pathways. Neurological disease becomes evident within 7 to 10 days of infection and develops to its ultimate severity in a matter of a few hours. The nature and severity of its signs, which include reluctance to move, dependent edema, proprioceptive and localized sensory deficits, tail and urinary bladder paralysis, gait abnormalities and limb paralysis usually confined to the pelvic limbs, depend upon the severity and location of the lesions which vary between individuals. The mortality rate is low in horses that do not become recumbent; most appear to recover completely. The disease may appear in an individual horse, as an explosive outbreak in a herd or stable, or multiple cases may occur over a period of weeks. When it occurs in bands of broodmares, respiratory disease in foals and abortions may occur concurrently (Bryans, unpublished observations; 24, 39). Death is usually a result of complications related indirectly to the primary lesion.

**EPIDEMIOLOGY**

The virus is distributed world-wide in domestic horses and has been identified as a pathogen of other Equidae kept as zoo animals (33). All three forms of disease have been described from most countries in which the infection has been identified. Two antigenically related subtypes (S-1 and S-2) are now known to infect horses (2, 8, 27, 32, 36). Although the DNA’s of the viruses exhibit less than 20 per cent base pair homology (4), they are antigenically related through at least four of their six major envelope glycoproteins (44) which are the viral proteins critical to infectivity and the protective immune response of the host. Primary infection by either virus elicits short-lived convalescent immunity to reinfection by homologous virus. Cross-immunity can result from multiple infection by either virus (3). Primary infection of immunologically naïve individuals, which are usually weanling foals, by either virus results in respiratory disease. Reinfection of the respiratory tract which leads to the occurrence of abortion or encephalomyelitis rarely presents noticeable signs. Both subtype viruses (Allen, unpublished observations) produce leukocyte cell-associated viremia (5) and abortion. The S-1 virus appears to possess characteristics
of enhanced virulence (10) as compared with S-2. It is found responsible for the
great majority of abortigenic infections and is the only type which has been isolated
from horses with neurological disease (3). The S-1 virus, in addition to antigenic
differences which are demonstrable by a number of techniques, infects a wide
variety of cell cultures (9), is adaptable to hamsters (Cricetus auratus) (14), has been
reported to exhibit endothelial cell tropism (32) and, according to the results of re­
striction endonuclease fingerprinting analysis (2), is more genetically stable than S-2.
In addition to its greater genetic variability, the S-2 virus is responsible for most
outbreaks of respiratory disease and for a comparatively small number of abortions
(3). Its cell culture host range is restricted apparently to cells of equine and porcine
origin; it replicates less efficiently than does S-1 in organ cultures prepared from
equine trachea, in the nasopharynx of intact immunologically experienced horses or
in the organs of infected fetuses (10).

Whether this observed association of viral subtypes predominantly with one or
more of the disease syndromes known to be produced by EHV-1 is controlled by, as
yet undefined, biological properties which may be peculiar to the subtype viruses,
or whether it is a pattern of disease influenced by immunity which has developed as
a result of the relative distribution of subtypes among populations, cannot yet be
known. Although the S-2 virus appears to be less virulent than S-1, it remains con­ceivable that both subtypes have inherently equal pathogenic potential and that the
observed differences in the patterns of disease produced are influenced by a more
effective immunity against secondary manifestations of the infection, i.e. abortion
or neurological disease, than presently available data can define.

As is the case for most herpesviruses, there is evidence (3, 11, 23) that establish­
ment of latent infections by EHV-1 is an important mechanism for survival of the
virus. Immunity, as defined by resistance of horses to deliberate challenge infection
by the same viral subtype and strain by which they were previously infected, lasts
for only a relatively short period of time (15, 18, 19, 20). It is demonstrable by in­ability to recover virus from either the upper respiratory system or leukocytes of
horses inoculated via the respiratory route and failure of a secondary humoral
immune response to occur.

Whether abortigenic infection of the fetus or neurological disease will occur in
horses in which infection of the upper respiratory system can be demonstrated is
unpredictable. It has been observed in the field as well as from investigations involv­
ing pregnant mares deliberately and repeatedly exposed (15) that, although abor­tions may occur in successive years among mares on individual farms, abortigenic
infection rarely occurs more than once in the same individual (7, 18). The immune
mechanisms which condition the putative enhanced degree of resistance to such
secondary disease in those animals which contract upper respiratory infection but
escape infection of the fetus or clinically evident disease of the central nervous
system have not been elucidated.

Restriction endonuclease analysis of more than 300 isolates (2) of EHV-1 sub­
types isolated from aborted fetuses and from horses with respiratory and neurologi­
cal disease in Kentucky during a period of twenty-two years have allowed recogni­
tion of, in addition to two laboratory manipulated live virus vaccine strains, 14 S-1
virus genotypes of which two, labeled P (prototype) and B (variant), represent the
cause of more than 90 per cent of abortions. The B variant virus was first detected
among S-1 viruses isolated during the period 1970-74; it became the dominant
genotype isolated during the years 1981 and 1982, but its incidence has since declined render­
ing the P genotype again the dominant strain of virus (1; Allen, unpublished observations). There are major differences in the electrophoretic profiles of the structural proteins of the subtype viruses and the viruses are readily distinguishable by the use of polyclonal antiseras in either reciprocal or kinetic neutralization tests. Such tests are not able to distinguish intrasubtypic antigenic differences among viral strains the DNA’s of which are readily distinguishable by electrophoretotyping. The existence of such intrasubtypic differences has been demonstrated by the use of monoclonal antibodies (47). The epidemiological significance of this antigenic variation or its possible effects upon the biological properties of the virus which control such important qualities as virulence and antigenicity are not yet known.

The first indication of antigenic diversity among strains of EHV-1 came from observations reported from Japan in 1959 (40). The data suggested that two strains of virus isolated from aborted fetuses in Hokkaido differed antigenically from the Kentucky D strain. Six years later, the results of a serological survey utilizing one of the Japanese strains of virus, now known to be S-2 and a Kentucky strain now known to be S-1 suggested that, although horses in many countries appeared to have been infected by both serotypes, those in certain others were infected predominately by one (30). Three of the four countries for which predominantly S-2 reactions were observed, of which one was New Zealand, are located in the Far East. Although Australian horses were not included in this survey, consideration of the history of EHV-1 disease in Australia in the same geographic area, presents an especially interesting viewpoint from which an improved understanding of the significance of viral subtypes in the evolution of herpesviral disease of the horse may be developed. Although EHV-1 had been isolated from the respiratory tract of diseased Australian horses as early as 1962 (22), herpesviral abortions were not recorded until 1975 (38) and no epidemic of abortigenic disease was observed until 1977 when multiple cases of abortion, neonatal deaths and infection of newborn foals were reported from New South Wales (13). Although the subtype of the 1975 isolate is unknown, the viruses isolated from the abortion “storms” which occurred in 1977 were found to be S-1 viruses (37) and the majority (42) of viruses recovered from aborted fetuses and examined since have been found to be S-1 strains. These data suggest that Australian horses were not infected by other than the S-2 virus prior to introduction of an S-1 virus which produced the more virulent form of disease in 1977. They also warn that these ecological conditions may exist in other countries, a circumstance which would place their populations at risk to serious losses in the event of introduction of S-1 virus.

**DIAGNOSIS**

Respiratory disease caused by EHV-1 presents no pathognomonic signs and may not be reliably distinguished by physical examination from the several other infectious diseases which affect the respiratory system of horses. Definitive diagnosis requires isolation of virus which may be accomplished by taking samples from the nasopharynx during the acute stages of the infection. Satisfactory samples are readily obtained by the use of a gauze swab held in a flexible instrument which may be constructed from stainless-steel wire. Samples taken in this manner should be immersed in a sterile transport medium, refrigerated, and processed by a laboratory within a few hours. If the time between sampling and laboratory examination is prolonged beyond a few hours, samples should be frozen. The virus may also be isolated from the leukocyte fraction of the blood. For this purpose, a sterile sample of
venous blood, using citrate as an anticoagulant, should be refrigerated and delivered to the laboratory. If it is necessary to freeze the sample, the leukocyte fraction should first be concentrated from and suspended in a reduced amount of the plasma.

Retrospective diagnosis of herpessvirial respiratory disease may be obtained by a variety of serological tests employing acute and convalescent sera. The results of such tests are most easily interpretable in the case of foals experiencing their initial infection. Because of the length of the incubation period between infection and appearance of disease, sera taken from mares that have aborted or from horses during the acute stages of neurological disease usually contain maximal amounts of antibodies. The results of tests using such sera are difficult to interpret unless samples are taken from clinically unaffected cohort members of a herd when the disease is first recognized and increased antibody titers are demonstrable in sequential samples from the same animals later in the course of an epidemic.

Fetuses aborted as a result of infection by EHV-1 present diagnostically typical gross lesions. The aborted fetus succumbs as a result of suffocation immediately before or during the process of delivery. Its foot pads are stained by meconium and it displays no post-mortem autolytic changes. The lungs are edematous and the pleural cavity often contains an excessive amount of clear transudate. The liver may contain small areas of necrosis, the thymus is frequently grossly necrotic and petechiae and ecchymotic hemorrhages are scattered over serosal surfaces. Typical herpetic intranuclear inclusion bodies, the pathognomonic lesion, are most frequently demonstrable in the epithelium of small bronchi and in hepatic cells at the periphery of areas of necrosis which are most commonly found near the portal triads. The virus may be readily isolated from the lung, thymus, liver and spleen. Culture of the mare is not reliably diagnostic. If the fetus is presented normally, the reproductive tract of the mare is rarely damaged. Mares which abort recover as efficiently as from normal parturition and their future reproductive ability is not compromised.

The initial signs of neurological disease caused by EHV-1 are a reluctance of affected horses to move. This is caused by the occurrence of proprioceptive defects consequent to the development of multiple lesions in the spinal cord. The severity of the disease depends, as stated, on the extent and distribution of the lesions. Although the occurrence of abortions concurrently with neurological disease in herds of pregnant mares provides a clue to the etiology of the latter disease, the fetus of pregnant mares suffering herpessvirial neurological disease may escape infection. Herpesviral neurological disease usually affects more than one horse in a herd; the initial case is commonly mistaken to be a result of trauma or ascribed to such causes as forage poisoning, segmental (protozoal) myelitis, or the "wobbler" syndrome. Ante-mortem diagnosis can be accomplished in many cases by isolation of the virus from the nasopharynx or the buffy coat of the blood. The gross lesions demonstrable at necropsy consist of a varying number of small plum-colored areas of degeneration in the spinal cord. Microscopically, vasculitis with endothelial swelling, necrosis of vessel walls, perivascular cuffing and pronounced axonal swelling are demonstrable. The vascular lesions are not confined to the central nervous system, but are demonstrable in other organs as well. Inclusion bodies are not usually found but application of fluorescent antibody techniques may demonstrate viral antigen in epithelial as well as endothelial cells of various organs including the vasculature of the spinal cord (34). Attempts to isolate virus from autopsy material are, more often than not, fruitless.
Because recognition of the pathogenic potential of the subtype virus allows useful epidemiological prognostication as well as a possible opportunity to adapt procedures for control of spread of the infection, especially from cases of respiratory disease in young horses to pregnant broodmares, it is important that subtypes be identified when viruses are isolated. This may be accomplished by the use of either specific polyclonal or monoclonal (47) antibody reagents as well as by DNA fingerprinting techniques (2, 36).

CONTROL

Equine herpesvirus 1 apparently exists wherever there are Equidae. The virus persists in nature as a result of its ability to cause latent infections which may lead to repeated instances of virus multiplication and shedding, as a result of its ability to reinfect horses that have experienced infection, and as a result of primary infection of immunologically inexperienced new additions to herds. No practically applicable methods are yet available to identify the existence of latent infection.

In the context of this epidemiological situation, control of disease caused by EHV-1 can concentrate on prevention of introduction of the S-1 virus into a population in which only the S-2 virus is present. It is now possible, as might have been the case for Australia (37) and perhaps Japan (26), to identify the absence of S-1 viruses in a population if respiratory infections, abortions and cases of neurological disease in the population in question are routinely monitored virologically. Unfortunately, however, if international competition and commerce in horses is to be maintained the problem of prevention of introduction of S-1 virus into susceptible populations appears to be practically impossible to solve.

The most efficient means available for control of diseases caused by EHV-1 are those which can be applied at the level of individual herds. They include institution of management practices which avoid as much stress as possible: separation of foaling mares into groups as early as possible in pregnancy, gentle practices of weaning foals and separation of weanlings, yearlings and all other horses from the pregnant mares. Outbreaks of herpesviral abortion and neurological disease most often occur under conditions of overcrowding, among bands of horses from which individuals are removed and frequently added, and among bands of pregnant broodmares to which new pregnant mares from sales are introduced. Although it is not feasible for government authorities to quarantine imported pregnant broodmares for prolonged periods, farms receiving such mares should sequester them from other pregnant mares until they have foaled. Horses of any age that are introduced to breeding farms should be quarantined not less than 21 days before they are mixed into the farm population.

International regulations which seek to control import of equine rhinopneumonitis by requiring that horses designated for import should not have displayed any signs of equine viral rhinopneumonitis nor have been on premises where such infections have occurred for three months prior to their exportation, except in those cases in which the disease can be recognized by occurrence of abortions or neurological disease, are inefficient for prevention of the introduction of disease unless they are reinforced at the level of individual destination herds by the quarantine measures suggested above.
Any approach to the control of most viral diseases must depend to a large extent upon development of a safe and immunogenically potent method of vaccination. Although it is conceivable that abortion or neurological disease caused by EHV-1 may result from systemic spread of endogenous virus in a latently infected individual, there is no evidence to suggest that epidemic disease caused by this virus spreads by means other than the respiratory route. Horses which contract such disease have had one or more previous infections by the virus which have not, except for a short period of time, provided them with either resistance to reinfection of the respiratory tract from which virus may spread to the susceptible fetus or with a capacity to prevent the presently undefined sequence of events which result in the particular form of neurological disease that may result from their infection by EHV-1. It is, therefore, clearly apparent that the key to prevention of disease caused by EHV-1 is to provide means to stimulate and maintain a quality of immunity which will prevent infection or reinfection of the respiratory tract.

There have been a number of attempts to produce vaccines to accomplish this purpose during the last thirty years. Chemically inactivated vaccines have been produced from virus obtained from infected equine fetal tissues, and from S-1 virus propagated in hamsters (18) and cell cultures of equine origin (6). Modified live viral vaccines have been produced from infected hamster tissues (18), from virus attenuated by extensive passage in porcine cell cultures (32) as well as from virus propagated in a cell culture of simian origin (35). Four of these vaccines have been licensed for use by veterinarians, two are no longer available. Of the latter, one was withdrawn because of an association between its use in horses and the occurrence of epidemic neurological disease (41) and the second was replaced by a chemically inactivated vaccine produced in cell cultures of equine origin (6, 7). Of the vaccines presently available in various countries, two are live virus vaccines which contain a genetically unique (3) S-1 strain of virus modified by extensive passage in porcine cells and rabbit cells. One product marketed in Europe contains, in addition to EHV-1 (S-1), two types of equine influenza virus as well as other viruses purported to be important causes of equine respiratory disease (43).

None of the vaccines presently licensed and distributed contain other than S-1 strains of EHV-1. Both the viral component of the modified live virus vaccine licensed in the United States for use as an aid in prevention of respiratory disease, and the virus with which the chemically inactivated vaccine licensed for use in prevention of both respiratory disease and abortion is prepared, have been shown by restriction endonuclease electrophoretyping of their DNA's to be genetically unique S-1 viruses. The inactivated vaccine is the only one that has been subjected to efficacy testing by controlled vaccination and challenge of pregnant mares. It is also the only contemporarily available vaccine subjected to an estimate of efficacy as a result of field use in a significantly large population of horses in which thorough evaluation of all causes of fetal wastage has been monitored by necropsy and virological examinations (3, 7). The use of this vaccine in approximately 65 per cent of the pregnant thoroughbred broodmare population of central Kentucky appears to have resulted in decreasing the incidence of EHV-1 abortions to less than half the lowest incidence of the disease ever recorded in this closely monitored population (2). It has been proven to be safe for use in horses of all ages and its use as an aid in the prevention of spread of the disease caused by EHV-1 can be recommended.
When abortion or neurological disease caused by EHV-1 is recognized among horses in a herd or stable, there is always a question as to whether unaffected members of the herd should be moved to prevent their becoming infected. In some cases the virus has spread to infect most members of the herd by the time that the first abortion or case of neurological disease occurs. The "die is cast" in such cases and moving horses will be profitless. Since one cannot know whether the disease has spread, however, if it is possible to remove as yet unaffected animals from the vicinity of an infected individual and to keep such animals in a quarantine situation away from other susceptible horses, they should be moved. Abortigenic infection is more likely to spread to other cohort mares at the time of abortion if the abortion occurs in a paddock or field than if it occurs at the time that a mare is confined in a loose-box. If the horses in contact with an infected individual have not been vaccinated, it is advisable to administer vaccine immediately upon recognition of the disease not only to the animals in immediate contact but to all other horses on the immediate premises. Infected fetuses, if not submitted to a diagnostic laboratory, should be disposed of along with all bedding and feed in the stall, by burning. The stall should be thoroughly cleaned and disinfected and left empty for a period of at least three weeks.

ACKNOWLEDGEMENT

This review is written in connection with projects of the Kentucky Agricultural Experiment Station and is published as paper No. 86-4-130 with permission of the Director of the Station.

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REFERENCES


