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EQUINE ARTERITIS VIRUS

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ABSTRACT

Equine arteritis virus (EAV) is a small, enveloped, positive-stranded RNA virus, in the family Arteriviridae, which can infect both horses and donkeys. While the majority of EAV infections are asymptomatic, acutely infected animals may develop a wide range of clinical signs, including pyrexia, limb and ventral edema, depression, rhinitis, and conjunctivitis. The virus may cause abortion and has caused mortality in neonates. After natural EAV infection, most horses develop a solid, long-term immunity to the disease. Mares and geldings eliminate the virus within 60 days, but 30 to 60% of acutely infected stallions will become persistently infected. These persistently infected animals maintain EAV within the reproductive tract, shed virus continuously in the semen, and can transmit the virus venereally. Mares infected venereally may not have clinical signs, but they shed large amounts of virus in nasopharyngeal secretions and in urine, which may result in lateral spread of the infection by an aerosol route. The consequences of venereally acquired infection are minimal, with no known effects on conception rate, but mares infected at a late stages of gestation may abort. Identification of carrier stallions is crucial to control the dissemination of EAV. The stallions can be identified by serological screening using a virus neutralization (VN) test. If positive at a titer of ≥ 1:4, the stallion should be tested for persistent infection by virus isolation from the sperm-rich fraction of the ejaculate, or by test mating. Shedding stallions should not be used for breeding, or should be bred only to mares seropositive from a natural infection or from vaccination; the mares should be subsequently isolated from seronegative horses for three weeks after natural or artificial insemination. A live attenuated (ARVAC) and a formalin-inactivated (ARTERVAC) vaccine are available. Both vaccines induce virus-neutralizing antibodies, the presence of which correlates with protection from disease, abortion, and the development of a persistent infection. Serological investigations indicate that EAV has a worldwide distribution and that its prevalence is increasing. As a consequence, an increasing number of equine viral arteritis (EVA) outbreaks is being reported. This trend is likely to continue unless action is taken to slow or halt the transmission of this agent through semen.

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Key words: equine arteritis virus, prevention, control, review

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INTRODUCTION

Equine viral arteritis is a disease which has been recognized in horses since the later part of the 19th century and has been referred to as "epizootic cellulitis pinkeye" or "equine influenza" in early veterinary literature (17, 91). The equine arteritis virus strain Bucyrus owes its name to the town in Ohio where it was first isolated during an abortion storm in 1953 (31, 32). During this outbreak, EVA was transmitted by the respiratory route, however, after the 1984 outbreak in Kentucky, a carrier state in stallions was identified (100). A third (36%) of the stallions that seroconverted during the outbreak were found to be persistently infected (99, 100). Further research demonstrated that persistently infected stallions shed virus continuously in semen and that a venereal infection occurred in mares bred by these stallions (59, 99, 101, 102, 103). This finding was the first direct confirmation of the assumption made as early as 1888 that there was an infectious agent present in the seminal fluid of certain stallions which was transmitted venereally to mares (5, 17, 91). Mares bred to shedding stallions can further disseminate the virus during the acute stage through aerosol transmission. In the past, disease outbreaks have been identified infrequently, however, with increased international travel of horses for competition and breeding, and shipment of semen for use in artificial insemination, the risk of virus transmission has increased and the number of confirmed outbreaks has risen. The purpose of this article is to review recent research on EAV, and to provide information about strategies for clinical diagnosis, virus detection, disease prevention and control.

THE VIRUS

Equine Arteritis Virus is a small, enveloped, single-stranded, positive-sense RNA virus. The virion has a diameter of 50 to 70 nm (7, 62) and consists of an isometric core (35 nm) surrounded by an envelope which contains small surface projections (30, 53, 81). EAV was originally classified as a non-arthropod-born member of the Togaviridae on the basis of virion morphology and size (90, 112). The finding of striking similarities with corona viruses and toro viruses in genome organization (25) and replication strategy (28, 107, 108) has recently resulted in classification of EAV as the prototypic member of a new family, the Arteriviridae (10), within a proposed order, the Nidovirales (nested set viruses). Other members of this new family include the porcine respiratory and reproductive syndrome virus (PRRSV), the simian hemorrhagic fever virus (SHFV), and the murine lactate dehydrogenase-elevating virus (LDV).

The EAV genome contains 7 open reading frames (ORFs) (25) (Figure 1). The 5' terminal 3/4 of the genome contains large ORF (ORF 1a/1b), encoding proteins involved in viral replication (25, 26, 94, 95). The 3' 1/4 of the genome contains 6 small ORFs, numbered 2 to 7, at least 4 of which encode structural proteins. The nucleocapsid protein (N) is encoded by ORF 7 (29, 109) while ORFs 2, 5, and 6 encode the viral membrane proteins, GS, GL, and M respectively (29) (Figure 2). The functions of the proteins encoded by ORFs 3 and 4 are currently unknown. The GL, M, and N are the predominant proteins in the virion, comprising ≥ 98% of the total virion protein content (29).

One serotype of EAV has been recognized using convalescent equine sera in cross neutralization and complement fixation (CF) tests (36, 113) but differences exist in both

pathogenicity and antigenicity between virus isolates. Antigenic variation can be detected amongst isolates in response to neutralization with polyclonal equine antisera and with monoclonal antibodies (MAbs) recognizing the GL protein (36, 41, 45, 113). Nucleotide sequence comparison of EAV M, N, and GL gene sequences (3, 13, 45, 88, 97) confirms that genetic variation exists between different virus isolates. Comparisons of the deduced amino acid sequences from geographically distinct isolates has shown between 1 and 6% heterogeneity for the N protein, 0 and 7% for the M protein (13, 97), and 0.4 and 10.2% for the GL protein, with one variable region in GL identified between amino acids 60 and 122 (3, 45). A large percentage of the substitutions found, outside of the one variable region found in GL, are conservative in nature, suggesting that only minimal variation can be tolerated in amino acid sequences of the structural proteins. Differences can also be demonstrated in pathogenicity (6, 71) and tissue culture growth characteristics (Glaser, unpublished data). Nothing is yet known about the biological significance of the demonstrated antigenic and genomic sequence heterogeneity.

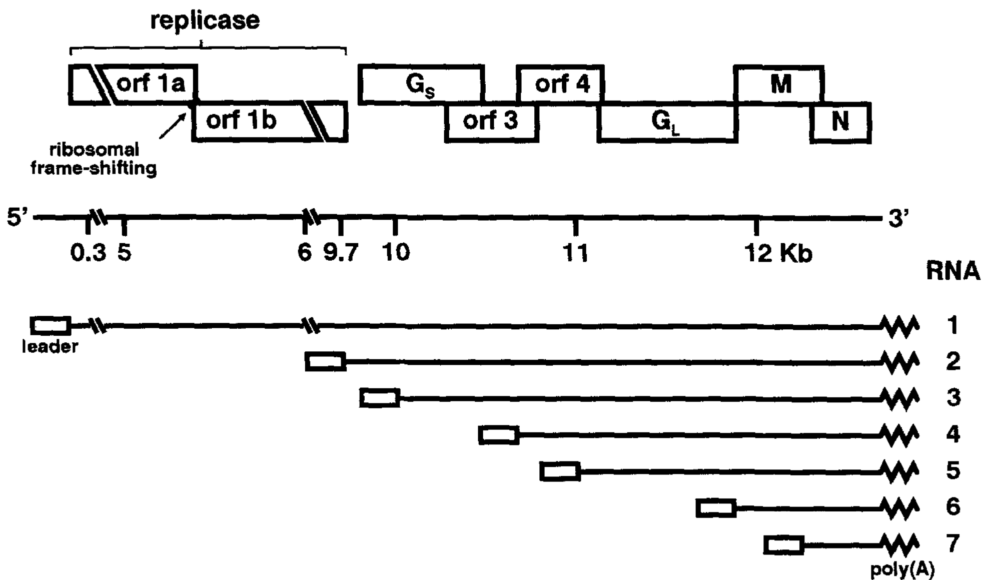

Figure 1. A schematic representation of the genomic organization and replication strategy of the Equine Arteritis virus. The position of the protein coding regions on the genome is shown on the top line, with the gene products encoded indicated within the blocks. A map of the genome, shown in kilobases is also shown. Proteins are translated from a series of messenger RNAs which share common sequences at each end.

There is evidence that the GL and N proteins are the major viral antigens against which an humoral immune response is directed. First, convalescent equine sera react consistently with the GL and N proteins, but almost never react with the M or GS proteins (14, 15, 16). Secondly, murine MAbs produced after immunization with virions have been shown to react against N and GL, but not against M or GS (1, 2, 11, 27, 45). In addition, all MAbs with neutralizing activity whose specificity has been determined, react with GL (1, 2, 27, 45) and peptides derived from the ectodomain of the GL protein induce neutralizing antibodies in mice, rabbits, and horses (2, 14). As a consequence, these genes and their products have been widely studied and are being developed as antigens for use in diagnostic tests and vaccines (see below).

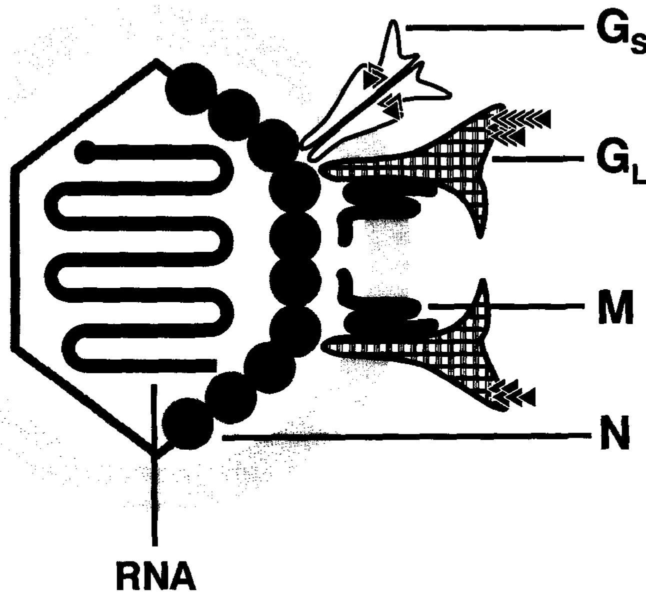

Figure 2. Model of the EAV virion. The virion RNA and nucleocapsid protein (N) form an isometric nucleocapsid, which is enclosed in a lipid envelope which contains at least three membrane proteins. The GL protein is the major glycoprotein in the virion and has been identified as a target for neutralizing antibodies.

THE DISEASE

Prevalence

Serological and clinical evidence indicates that EVA has a worldwide distribution and seroreactivity has been reported in horses, donkeys, mules, and zebras (51, 77, 79, 80, 85, 86, 87, 88, 89, 111). The percentage of horses which are seropositive varies among countries and breeds. In general, Standardbreds have a high, while Thoroughbreds have a low seroprevalence (54, 105). It is not known if the differences in seroprevalence reflect different susceptibilities to infection or are merely the consequence of different risks of exposure. A recent serological surveys in Germany (34) indicated that the number of seropositive horses has increased from around 2% in 1988/89 to over 20% in 1995; increased seroprevalence has also been reported in Sweden (57) and Italy (8). It therefore seems that within certain equine populations, the exposure to EAV has increased substantially in the recent past.

Until recently EAV had only been isolated from horses. However, Paweska and co-workers (85, 86, 88), following up on the detection of serum antibodies to EAV in donkeys (51, 79) demonstrated that these animals were susceptible to infection as well. The asinine virus was transmitted by both the venereal and respiratory routes and is closely related at the nucleotide sequence level to EAV isolates from horses. Additionally, an equine isolate of EAV has been used to experimentally infect donkeys (77), providing evidence that EAV has a host range which may include all equids.

Pathogenesis and Clinical Signs

After aerosol exposure, EAV replicates initially in pulmonary macrophages, and rapidly spreads throughout the body and infects several other cell types, including endothelial cells in all components of the circulatory system (23), smooth muscle cells in arteries and the myometrium (61, 110), renal tubular epithelium, as well as in the adrenal epithelium and, infrequently, in the liver parenchyma, intestinal crypt cells, and bronchial epithelium (23, 61). In experimental infections with the Bucyrus isolate, the appearance of fever, depression, and edema, coincided with the presence of viral antigen within the endothelia of veins and lymphatics (23).

The consequences of an EAV infection range from a subclinical course, only recognized by seroconversion (21, 69, 106), to severe illness (32). In natural outbreaks, clinical signs can be variable but are generally mild. Signs may include pyrexia, depression, anorexia, edema of scrotum, ventral trunk, and limbs, stiffness, conjunctivitis, lacrimation, serous nasal discharge, and urticaria (18, 21, 32, 44, 73, 98, 102). Severe clinical signs include respiratory distress, weakness, ataxia, and colic. Neonates and foals can present with an acute onset of respiratory distress as a result of a rapidly progressive bronchointerstitial pneumonia or with a pneumoenteric syndrome, which can be fatal (9, 47, 106). Severe signs and/or mortality are rare in adults except after experimental infection with some variants of the Bucyrus isolate. Clinical signs typically begin 2 to 14 days following aerosol exposure and persist for 2 to 9 days (73, 105). In the case of venereal exposure, the incubation period is 6 to 8 days (105). Virus is shed in nasopharyngeal secretions and urine and is present in serum and in the buffy coat. Virus can generally be isolated from the

nasopharynx and the buffy coat for 3 to 19 days post infection (68). Adult mares and geldings generally make an uneventful recovery and are resistant to clinical disease after re-exposure for perhaps their lifetime (44, 66, 67). Stallions recover clinically, but 30 to 60% will become persistently infected (see below.). The ratio of subclinical to clinical infection in reported outbreaks where infection was spread by the respiratory route has varied from 0.4:1 to 0.8:1 (21, 114), while after venereal transmission ratios varied between 1.4:1 to 6:1, depending on the stallion involved (99).

Abortion

Another possible sequel to an EAV infection is abortion, which may occur even if the mare had no clinical signs. In cases of natural exposure, the abortion rate has varied from less than 10% to more than 60% (18, 32, 34, 46, 48, 56, 73, 99) but abortion rates after experimental infection can be higher (20). Abortions have occurred in mares from 3 to 10 months of gestation (20, 99) and most often occur during the late acute or early convalescent phase of infection (18, 33, 48). The fetus is usually expelled partially autolysed, but may be expelled fresh and gross lesions are not common (33, 56, 105). When lesions are present, they can include congestion and edema of the mediastinal tissues and lymph nodes, epicardial hemorrhages, petechial hemorrhages on pleural and peritoneal surfaces and small amounts of clear fluid within the pleural cavity (33). Histopathological lesions are rarely reported, with the exception of one incidence in which fetuses aborted as a result of EAV infection had widespread necrotizing vasculitis in many tissues, including liver, spleen, lung, and brain (5). Virus can readily be isolated from the placenta and tissues and body fluids of the fetus, so these may serve as a source of virus which can infect other susceptible animals.

The pathogenesis of EAV induced abortion is not yet understood and it is unclear what the respective roles are of maternal versus fetal infection. The severe necrotic myometritis which was found in the uterus of mares after experimental infection led to the hypothesis that abortion was due to decreased fetal support caused by mechanical compression of blood vessels as a result of edema and to decreased uterine progesterone production (19). The presence of viral antigen has been demonstrated within smooth muscle cells of the myometrium (110), providing direct evidence that inflammation seen in this organ is due (in part) to the presence of replicating virus. Recent immunohistochemical identification of viral antigen within areolar trophoblast (24), together with the evidence of myometrial infection, suggests that abortion may result from the direct impairment of maternal-fetal support and not from fetal infection. This hypothesis is further supported by the lack of lesions in the fetus (20, 33, 48, 49, 84, 110) and the inability to demonstrate the presence of intracellular viral antigen in fetal tissues (24) which suggests that replication of virus within the fetus prior to abortion does not occur and therefore cannot play a major role in its pathogenesis. However, the one report of widespread vasculitis in two fetuses aborted as a result of EAV infection (56) indicates that there may be strain differences in the ability of the virus to cause fetal lesions and insufficient evidence exists to rule out a role for fetal infection in abortion. It is possible that a combination of pathogenic mechanisms involving both maternal and fetal components are involved in abortion. Which, if any, component plays a predominent role is yet to be determined.

Persistent Infection

While mares and geldings are able to eliminate virus from all body tissues by 57 days post infection, 30 to 60 % of stallions become persistently infected (76, 82, 100, 101). In these animals, virus is maintained in the accessory organs of the reproductive tract, principally the ampullae of the vas deferens (40, 82), and is shed constantly in the semen (59). Three carrier states exist in the stallion: a short-term state during convalescence (duration of several weeks), a medium-term carrier state (lasting for 3 to 9 months), and a long-term chronic condition (82, 99, 100, 105) which may persist for years after the initial infection. The development and maintenance of virus persistence is, in large part, dependent of the presence of testosterone (52, 59). Persistently infected stallions who were castrated but were given testosterone continued to shed virus, while those administered a placebo ceased virus shedding (59). In addition, efforts to establish long-term persistence in prepubertal colts before the appearance of detectable concentrations of circulating testosterone, or to induce persistent EAV infection in castrated male horses, have been unsuccessful (42, 52, 76). However, virus could still be isolated from the accessory glands of the reproductive tracts of prepuberal colts up to 120 days post infection (52), while virus could not be detected in geldings after day 57 (76). This suggests, that even in the absence of detectable levels of testosterone, the accessory sex glands of colts provide a more permissive atmosphere for viral persistence than those of geldings.

While the presence of testosterone appears to be essential for the establishment and maintenance of a persistent genital tract infection, the mechanism by which a stallion becomes a persistent semen shedder is unknown. Virus can be isolated from the reproductive tract and is shed in the semen of most stallions during an acute infection (82). The ability of a large percentage of stallions to effectively eliminate the virus in time suggests that differences in the immune response of the host may be involved, but there is as yet no empirical evidence available to support this suggestion. Alternatively, virus strains may have biological differences which influence their ability to persist in the reproductive tract. Establishment of persistence may involve a multifactorial process, with dependence on both host and viral factors, however, sexual rest following acute EAV infection may be important in reducing the likelihood of a stallion becoming a chronic EAV carrier (82, 103).

Recovery

After clinical recovery from the initial infection, there is no significant decrease in the fertility of shedding stallions (57, 83, 100, 101). Mares infected after service by a carrier stallion do not appear to have any related fertility problems during the same or subsequent years and there are no reports of mares becoming EAV carriers or chronic shedders, or of virus passage by the venereal route from a seropositive mare causing clinical disease or seroconversion in a stallion. Foals born to seropositive mares acquire through the colostrum maternal antibodies to EAV (70) which decline to extinction within 2 to 6 months. Previously infected horses are protected from clinical disease after infection with the virulent virus (67). Natural immunity has been

demonstrated to persist for up to seven years (44) and may persist longer. Presence of the neutralizing antibody correlates very well with protection from the systemic disease, abortion, and the development of persistent infection in stallions after aerosol or venereal exposure to virus (39, 40, 43, 74, 75, 105).

TRANSMISSION

The two major routes by which EAV is spread are by aerosols generated from respiratory secretions and urine from an acutely infected animal and by venereal infection of a mare by a virus shedding stallion (98, 99, 103, 105) (Figure 3). Close contact between animals is generally required for efficient virus spread in aerosol transmission. Personnel and fomites may play a minor

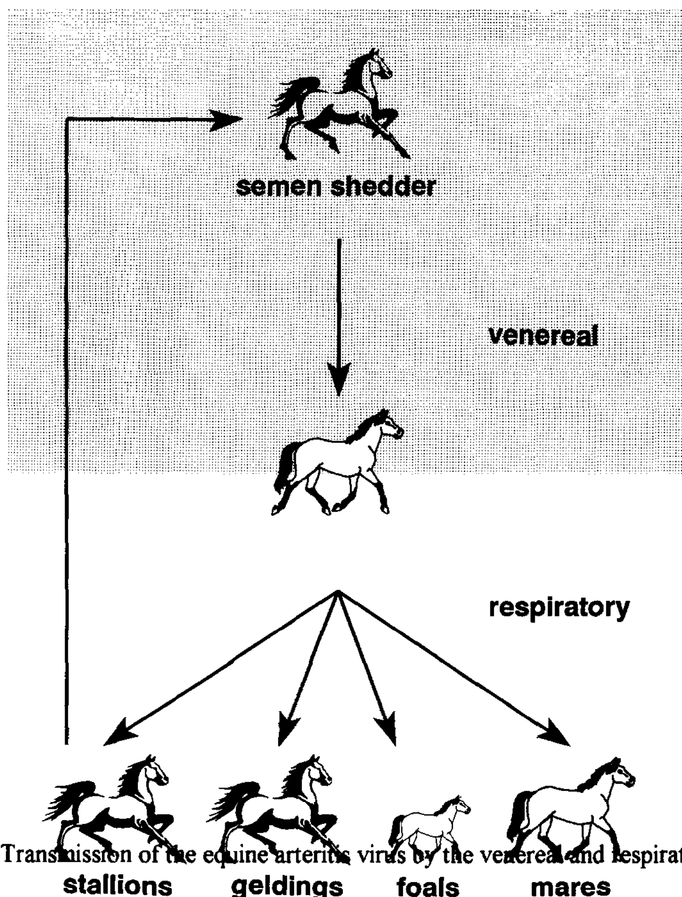

Figure 3. Transmission of the equine arteritis virus by the venereal and respiratory routes.

role in virus dissemination (21). Virus is viable in fresh, chilled, and frozen semen, and venereal transmission is efficient, with 85 to 100 % of seronegative mares seroconverting after being bred to stallions shedding virus (99). In several cases, outbreaks of clinical disease have been traced to a persistently infected stallion (105, 114). Mares infected venereally return home with an acute EAV infection, which is then spread horizontally to other susceptible animals in the area. Mares infected late in gestation may pass the virus vertically to their fetus (106). Foals with vertically acquired infection can be born weak or may appear normal. These foals usually present with an acute onset of severe respiratory distress within a few days of birth and infection can be lethal (24, 106).

DIAGNOSIS

Clinically, EVA resembles several other viral infections of equines, such as those caused by equine herpes virus types 1 and 4, equine rhinovirus, equine influenza virus, equine adenovirus, and equine infectious anaemia virus, so that a definitive diagnosis requires laboratory confirmation. Acute infections can be diagnosed by virus isolation or by serologically identifying a 4-fold or greater rise in neutralizing titer between acute and convalescent serum samples. In the case of abortion, virus isolation can be attempted from fetal and placental tissues or seroconversion can be demonstrated in the mare. Persistent infection in stallions can be diagnosed by first screening serum for antibody in a virus neutralization (VN) test. If seropositive at a titer of ≥ 1:4, virus isolation should be performed on the untreated, sperm-rich fraction of the ejaculate or the stallion should be test mated to seronegative mares who are monitored for seroconversion.

Antibody Detection

Several methods can be used to detect EAV-specific antibody in serum. In accordance with OIE guidelines, detection of EAV-specific antibody is currently based on a complement-dependent VN test (55, 93). The VN antibody titers develop 2 to 4 weeks following infection, are maximal at 2 to 4 months, and remain stable for several years (44). The endpoint for titer determination is that dilution of antibody which results in 75% protection of the monolayer from cytopathic effect using 100 tissue culture infective dose 50 (TCID50) units of virus. The standard test uses the Bucyrus isolate and RK-13 cells. A titer of 1:4 or greater in duplicate sera is considered EAV seropositive.

Comparisons of several of the currently used indicator viruses, including several Bucyrus isolates, in cross neutralization experiments reveal that differences exist in the sensitivity of these viruses to neutralization by convalescent sera from horses infected experimentally with heterologous isolates (113). With high initial titers against homologous virus, these differences are not very significant, but detection of lower titers with some of the indicator viruses might be problematic. The subjective endpoint and the use of different EAV strains and a variety of cell lines (with different passage history) for this test can result in discrepancies in titer values reported by different laboratories.

Antibodies to EAV can additionally be demonstrated by CF tests, and enzyme-linked immunosorbent assays (ELISAs) (6, 15, 16, 22, 36, 58, 63, 86, 89). The CF test is potentially useful for detection of recent infections as the CF titer peaks 2 to 4 weeks after infection and decreases below detectable limits after 8 months (36). However, most diagnostic facilities do not offer this test for the detection of EAV-specific antibodies.

The earliest ELISA tests developed used purified virus as a source of antigen (22, 58). The major problem with these ELISAs was the large number of false-positive reactions due to the presence of tissue culture derived antigens which reacted with antibodies, present in a large percentage of equine sera, acquired from tissue culture derived vaccines (22). Following the identification of the GL protein as a prominent antigen against which both neutralizing and non-neutralizing antibodies were produced (1, 2, 14, 27, 45), an ELISA based on the bacterially expressed GL ectodomain of EAV-Utrecht has been developed. The ELISA is sensitive and specific for the detection of EAV-specific antibodies in horse, donkey, and mule sera (15, 89) and can detect the presence of specific antibody before a positive VN response is detected (15). Absorbance values correlate well with neutralizing antibody titers. The ELISA can be used to detect differences between IgG and IgM antibody levels in donkeys and may have utility in distinguishing between recent and historical EAV infection. Another ELISA based on the GL protein has been developed which uses multiple ectodomain sequences from phylogenetically different isolates (de Vries et al., unpublished data). This test was developed because of the recent evidence that there is considerable amino acid sequence variation within this domain between virus isolates (3, 45). Since the same part of GL has also been identified as a dominant neutralization sensitive site (3, 45) and cross neutralization experiments (113) suggested that horses exposed to different EAV isolates produce antibodies with differential activities which might be (in part) attributable to variation within this region, the inclusion of multiple ectodomain sequences could theoretically improve the sensitivity of the test over the use of a single ectodomain sequence. When equine sera are tested in the ELISA against single, distinct ectodomain sequences, there is clear variation in the efficiency with which these sequences are recognized. An ELISA based on the N protein is also highly sensitive and specific (16) and may allow differentiation between the antibody response promoted by a subunit vaccine (14) from that due to infection. There are indications that the ELISA tests will be more sensitive than the VN test currently in use. The potential disadvantage of these tests is that while a positive VN test can be directly correlated with biological protection from disease, this might not be true of a positive ELISA test as non-neutralizing as well as neutralizing antibodies are detected.

Virus Detection

The current test for identifying virus in tissues and semen is virus isolation in cell culture (64, 65). Tissue extracts or diluted semen are incubated together with a permissive cell line in monolayer culture, often RK-13 cells, and the cells are observed for cytopathic effect. The virus can be positively identified by immunofluorescence or immunocytochemistry using specific polyclonal or monoclonal antisera (60) or by testing in a VN assay with defined reference antisera.

The isolation of EAV in cell culture from field samples of blood, semen, and tissues has proven problematic in some instances (71). The RK-13 cells are generally the most sensitive, but

the ability to consistently isolate virus is dependent on both the RK-13 cell subculture and its passage level (P.J. Timoney, personal communication). Proper handling of samples during collection and submission can also have a large impact on a successful outcome. If virus isolation fails to provide a positive answer, diagnosis can be made by testing acute and convalescent sera in a VN assay to determine if the animals seroconverted. Alternatively, virus transmission can be demonstrated by inoculation of blood from febrile horses into susceptible horses, followed by virus isolation from nasal swabs and blood of these experimentally infected horses, and by their subsequent seroconversion to EAV. In the case of stallions suspected of being persistently infected, test mating to seronegative mares and testing these mares for seroconversion will provide a definitive answer.

Additional possibilities for detecting the presence of viral antigen or RNA without the need for tissue culture exist. It is possible to directly identify viral antigen in formalin-fixed tissues using immunohistochemistry (61). This may be useful for establishing a positive diagnosis when only formalin-fixed material is available. The presence of viral RNA can be detected by RT-PCR, which can be performed on both tissue and semen (4, 12, 92, 96). Tests performed with two sets of nested primers in sequential reactions can be more sensitive than virus isolation for the detection of virus (4). Since the RT-PCR only detects RNA, no information would be available as to the potential infectious nature of the virus. This is especially relevant for the detection of virus in semen, as the limiting infectious dose has not been determined and little is known about the infectious status of semen which has virus detectible by RT-PCR but not by virus isolation.

Sample Selection

Acute disease. In cases of respiratory disease, EDTA, heparinized, or citrated blood should be taken as soon as possible after the first signs of illness. Nasopharyngeal and conjunctival swabs can also be submitted after submersion in a suitable viral transport medium. All samples should be chilled, and transported quickly to a competent diagnostic laboratory for virus isolation. Clotted blood should also be obtained at this time and 3 weeks later for use in demonstrating seroconversion. In the event of foal or adult animal death, samples from lung, liver, spleen, lymph nodes, and kidney should be submitted for virus isolation. If rapid transport of chilled samples is not possible, they should be stored at below -20°C and shipped frozen.

In EAV related abortion, both fetus and placenta contain large amounts of virus (20). Samples of placenta, spleen, lung, and kidney along with fetal and placental fluids should be collected in a sterile manner as soon as possible after the abortion occurred, chilled on ice, and submitted for virus isolation. Blood should be obtained from the mare at the time of the abortion and three weeks later for testing by VN.

Persistent infection. After having determined that a stallion is seropositive at a titer of $\geq$ 1:4 in a VN test, a semen sample should be collected using an artificial vagina or a condom and a phantom or a teaser mare. If this is not possible, a dismount sample can be collected at the time of breeding, however this is less satisfactory. The sample should be from the sperm-rich fraction of the full ejaculate and should be chilled immediately and shipped at 4°C to arrive at the diagnostic facility within 24 hours. If this is not possible, the sample should be frozen at below - 20°C and

shipped to the diagnostic facility under these conditions. Submission of two samples, collected the same day or on consecutive days is recommended. Washing of the penis with antiseptics or disinfectants prior to collection of the samples should be avoided. Alternatively, the stallion can be test mated to two seronegative mares, who are kept in isolation for 3 weeks and monitored for seroconversion.

PREVENTION AND CONTROL

Vaccines

The early studies of Doll et al. (33) demonstrated that virulent EAV could be attenuated by passage in tissue culture while retaining immunogenicity. Serial passage through horse kidney, rabbit kidney, and equine dermis cells have served to attenuate virulent virus (50, 66, 67, 72, 74, 75) resulting in a modified live vaccine (MLV) (105). The MLV does not produce any side effects in vaccinated horses apart from a short term abnormality of sperm morphology, and a mild fever with no overt clinical signs (104). However, live virus can be isolated sporadically from the nasopharynx and blood after MLV vaccination (37, 105). The VN antibody titers are induced within 5 to 8 days and persist for at least 2 years (74, 105).

The MLV protects against clinical disease and reduces the amount of virus shed from the respiratory tract in experimental infection. Horses in contact with, and mares served by vaccinated stallions are not infected by EAV (78, 105) and vaccinated mares experimentally challenged by artificial insemination are protected from clinical disease, but not from the infection (75). In the field the MLV vaccine (Arvac, Fort Dodge Laboratories) has been used to control EAV outbreaks in some states of the USA since 1984 but the vaccine is not licensed worldwide.

Fukunaga and co-workers (38, 39) have pioneered the use of a formalin-inactivated whole virus vaccine. Secondary immunization 4 weeks after primary vaccination resulted in VN antibody titers of up to 1:5120. The antibody titer decreased rapidly but a third immunization after 2 months resulted in a VN titer of between 1:80 to 1:320 persisting for 6 months. The 50% protective level of antibody was calculated at a VN titer of 1:43. However, even though clinical disease was averted, not all horses with high antibody levels were protected from infection since live virus was recovered from blood samples after experimental exposure. The inactivated vaccine prevents stallions from becoming semen shedders after exposure to EAV and protects pregnant mares from abortion (40, 43).

After the 1993 outbreak of EVA (114), an inactivated whole virus vaccine (Artervac, Fort Dodge Laboratories) has been available in the UK and Ireland. The vaccine induces high neutralizing antibody titers and produces no side effects but its efficacy in preventing clinical disease, persistent infection in stallions, and abortion after re-exposure to the virus is still unknown. Further testing of this vaccine is required before it gains a full product license in the UK.

Following the identification of major viral epitopes on the EAV GL protein (1, 2, 14, 45), viral subunits have been used to immunize animals in attempts to induce VN responses. Both a

GL-specific peptide (amino acid residues 75 through 97) and a bacterially expressed fusion protein (residues 55 through 98) have been shown to induce a VN antibody response in vaccinated horses (14), and a second GL-specific peptide (residues 93 through 113) (2) induced VN antibodies in mice. Protection studies on these subunit vaccines are awaited.

Management

The pivotal point in any prevention and control program is the identification of persistently infected stallions and the institution of management procedures to prevent the introduction of EAV into susceptible populations through the breeding of mares by natural service or artificial insemination. The best way to prevent EAV infection and its spread is for each country or breeding association to propose and enforce a code of practice to be followed by horse owners and breeders. In the UK the Horserace Betting Levy Board distributes an annual code of practice each November, which aims to help breeders minimize the risk of EVA in the horse population by making recommendations for disease prevention and control. The breeders themselves are responsible for implementing the code; breaches only come to light in the event of a disease outbreak.

Anyone intending to import horses, semen or embryos, should seek information from specialists about the incidence of EVA in the exporting country and in the individual farms; semen and embryo donors must be tested before importation. Imported animals should be placed in isolation immediately on arrival and kept under observation for 14 days during which time blood samples should be tested for EAV antibodies. As a basic first step to preventing disease individual owners should (i) serologically screen resident animals (the start of each breeding season is normally the best time) and (ii) request that animals moved onto their premises be tested before movement. If a stallion proves to be seropositive, he should not be used for breeding through natural service or AI until he is shown to be virus free. If shedding virus, stallions might still be used if certain restrictions are met. These stallions should be bred only to mares seropositive either through natural infection or vaccination, that are then kept in isolation for three weeks after breeding. Seropositive mares represent no risk to stallions or other horses, but antibody status could be confirmed by testing a second serum sample 2 to 3 weeks after the first if it is important to establish whether the infection was recent or historical. A stable titer would indicate historical infection, while a rising titer would provide evidence that the infection was more recent, i.e. within the last several weeks.

The option to use a shedding stallion in a breeding facility may depend on the regional regulations. Within the EU, no shedding stallions can be present on a stud which engages in the intercommunity trade of semen. In addition, semen and stallions imported to the EU must be certified as seronegative for EAV or if seropositive, have a negative virus isolation result from semen (35) The importation of semen from stallions shedding EAV is also prohibited. In the UK, EVA is a notifiable disease requiring the Ministry of Agriculture to be informed of all seropositive shedding stallions, of all mares infected by the venereal route (AI and natural mating), and of all infected animals during a disease outbreak.

During outbreaks the spread of EAV is best controlled by movement restrictions, isolation of infected animals followed by a quarantine period after recovery, good hygiene including allocation of personnel to deal solely with infected animals, and rigorous diagnostic surveillance of infected and in-contact animals. The option to use vaccination to control the spread of disease is also available in some, but not all countries. Following disease outbreaks (105, 114) and the subsequent imposition of vaccination policies, more stringent EAV control programs are generally implemented. These may include a requirement for annual vaccination of all non-carrier stallions and teasers prior to the breeding season and vaccination of seronegative mares served by seropositive carrier stallions. In countries which have vaccination policies for EVA, all animals should be tested prior to first vaccination and issued with a certificate to prove their original seronegative status.

CONCLUSION

Equine viral arteritis is entirely preventable if simple serosurveillance and hygienic procedures are followed by horse owners, breeders, and keepers. The establishment of an international code of practice covering all aspects of this disease (including AI and embryo transfer) and its enforcing by equine interest groups, or state veterinary services, would certainly help limit spread.of the disease. Such a code should clearly specify policy on diagnostic testing and dealing with persistent semen shedders and could include an option for vaccination. Controlling the further dissemination of EAV requires a concerted effort on the part of all those involved in the equine industry. Current international regulations have created an environment in which it is no longer possible to ignore the presence of this agent in semen. The straightforward epidemiology involved and the fact that the presence of neutralizing antibody correlates well with protection from disease, abortion, and the development of persistent infection in stallions is evidence that control programs, once instituted, are likely to be successful and indeed, have been successful.

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