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ScienceDirect
Theriogenology xxx (2008) xxx–xxx
Theriogenology
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Equine viral arteritis: Current status and prevention

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Abstract

Recently, there has been increased interest in equine viral arteritis (EVA) among veterinarians and horse owners. Outbreaks of the disease were identified initially in New Mexico, USA in 2006, and in the Normandy region of France in the summer of 2007. Both occurrences were associated with AI of cool-shipped semen. Each was linked to respiratory illness, neonatal death, abortion, development of carrier stallions, and cancellation of equestrian events. In light of the increased interest, this paper will present a brief case history, followed by a review addressing common concerns regarding EVA, current status, and control and prevention strategies, including vaccination, and recommended bio-security measures.

Keywords: Equine; Equine arteritis virus; Persistent infection; Transmission; Prevention

1. Case history

Early in the first week of June 2006 on an equine stud farm in New Mexico, USA, early pregnancy losses were detected in mares previously confirmed pregnant. The problem continued, and on June 16 the farm manager contacted the Maxwell H. Gluck Equine Research Center, University of Kentucky, to discuss probable causes [1]. Equine viral arteritis (EVA) was suggested as a likely cause of the abortions. Of the four stallions standing at this farm, the first developed fever, depression and dependant edema, especially of the scrotum and hind limbs. His fertility decreased, and he failed to impregnate any mares for the remainder of the season. Equine arteritis virus (EAV) was subsequently isolated from the semen of this stallion. Shortly thereafter, the stud manager reported that a second stallion had developed pyrexia; however, his fertility remained adequate to impregnate mares. Virus-positive semen was shipped from this stallion to multiple states within the United States prior to and during the second and third weeks of June and before the diagnosis of EAV infection by the Maxwell H. Gluck Center on June 26, 2006 [1]. During this time, two other stallions became infected. Stallion 3 developed a short-lived fever without substantial signs of disease, whereas Stallion 4 had neither fever nor clinical signs of disease. Both stallions subsequently tested positive for EAV in their semen. In early September 2006, Stallion 1 died from complications associated with laminitis. Stallion 2 remained persistently infected, shedding virus in his semen through January 31, 2007, tested negative on multiple virus isolation attempts in March 2007, and had three negative test breedings in July 2007. Stallion 3 tested positive through January 9, 2007, tested negative on multiple virus isolation attempts in February 2007, and had three negative test breedings in July 2007. Stallion 4 remains persistently infected, shedding

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doi:10.1016/j.theriogenology.2008.04.020

infectious virus in his semen as of December, 2007 [Murray J. personal communication]. Also associated with the outbreak were 30 abortions, involving approximately half of the exposed pregnant mares on the farm with the index case.

The virus was disseminated to farms in 18 states, either via cool-shipped semen (48 mares) or mare transport (20 mares and foals) before the spread was controlled by quarantine and close surveillance [1]. A substantial spread point occurred in Utah via cool-shipped semen transport and subsequent animal movement; this resulted in respiratory illness, abortion, neonatal pneumonia, and death [1]. These outbreaks were finally controlled by quarantines and close surveillance.

2. Introduction

An extensive outbreak of equine viral arteritis that occurred in Kentucky Thoroughbreds in 1984 generated widespread interest, publicity and concern [2–5]. A number of other outbreaks of the disease have since been reported from North America and Europe [5–10]. Similarly, equine arteritis virus infection of horses has been identified in countries including Australia, New Zealand, and South Africa, previously thought to be largely or completely free of the virus [11–14]. Serological surveys have shown that EAV infection occurs among horses in North and South America, Europe, Australasia, Africa, and Asia [15], with considerable variation in seroprevalence of EAV infection among countries and within equine populations in some countries.

In the summer and fall of 2006, related to the use of cool-shipped semen for AI, EVA was identified in New Mexico and five other states, in association with abortion, respiratory disease, neonatal illness, and the development of the carrier state in an additional number of American Quarter Horse stallions (numerically the largest equine breed in the USA) reported at the 2006 Annual Meeting of the United States Animal Health Association, ‘‘of overriding importance was the ease with which infection was very effectively spread among an immunologically naïve population through the use of semen from a stallion acutely and later, persistently infected with EAV. This occurrence of EVA was the first in which there was widespread dissemination of EAV in Quarter Horses, a breed essentially not previously exposed to this virus’’ [1]. Most recently, in the summer and fall of 2007, the disease occurred on breeding farms in the Normandy region of France including the national stud, the Haras du Pin, where the infection was first diagnosed in a Percheron stallion. Within a week, other stallions developed clinical signs of the disease. This outbreak, which was also linked to AI of cool-shipped semen, eventually affected 26 farms, resulting in illness, neonatal death, abortion, development of persistently infected stallions, and cancellation of equestrian events [16]. The following review will address some of the common concerns with regard to EVA, its etiology, epidemiology, pathogenesis, pathology, clinical findings, diagnosis, transmission risks, control and prevention, including vaccination and recommended bio-security measures.

3. Etiology

Due to the distinctive inflammation in the muscle wall of small arteries in the acute phase of the infection, the virus is called equine arteritis virus and the disease it causes, EVA [15–17]. Equine arteritis virus is the prototype virus in the family Arteriviridae (genus Arterivirus, order Nidovirales), which also includes porcine respiratory and reproductive syndrome virus (PRRSV), lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus [18,19]. It is an enveloped, single-stranded, positive-sense, RNA molecule (Fig. 1) [19,20]. There is only one known serotype of EAV, but geographically and temporally distinct strains of EAV differ in the severity of the clinical disease they induce and in their abortigenic potential [15,21–29]. Strains of EAV from North America and Europe share at least 85% nucleotide identity; following phylogenetic analysis, these viruses generally segregate into North American and European geographical groups.

Survival of EAV is temperature dependant; although it may survive only 20–30 min at 56 °C and from 2 to 3 d at 37 °C, it can survive up to 75 d at 4 °C. Tissue culture fluid or tissue samples containing EAV can be stored at −70 °C for years without loss of infectivity [30]. However, the virus is readily inactivated by lipid solvents (ether and chloroform) and by common disinfectants and detergents [31].

4. Epidemiology

Although EVA is a disease almost exclusively of equids, antibodies to EAV have been identified in donkeys in South Africa [12,13] and in the USA [32]. Clinical, virological and serological responses of donkeys have been determined following intranasal inoculation with the KY-84 strain of equine arteritis virus. Equine arteritis virus has also been reported associated with a case of abortion in an alpaca in

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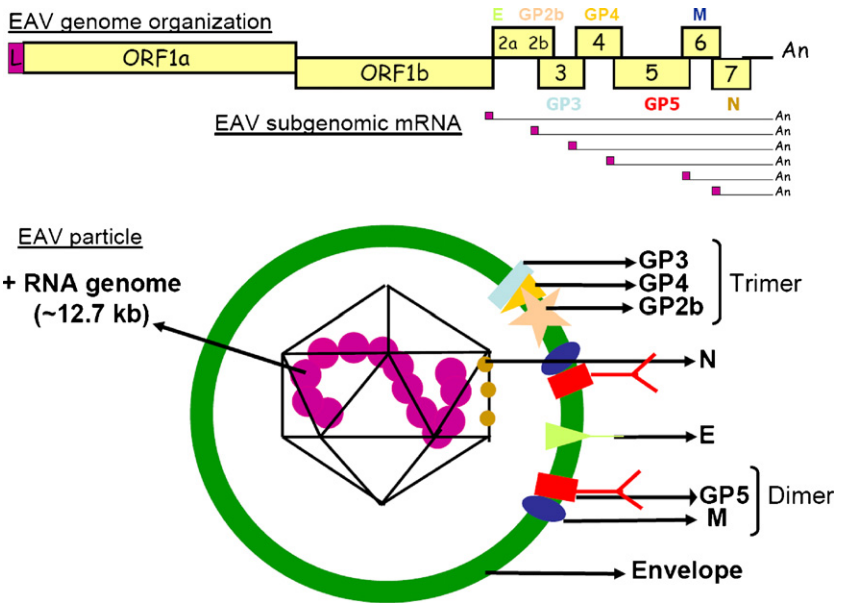

Fig. 1. Schematic representation of the EAV genome organization and the virus particle (adapted from [30]).

Germany, based on a positive reaction in the polymerase chain reaction assay [33].

Serological surveys have shown that EAV infection occurs among horses in North and South America, Europe, Australasia, Africa, and Asia [15], with considerable variation in seroprevalence of EAV infection among countries and within equine populations in some countries. In that regard, Iceland and Japan are apparently free of the virus, whereas EAV infection is relatively common in horses in several European countries. The seroprevalence of EAV infection was estimated at 11.3% in Swiss horses, and 2.3% in English horses, in studies conducted in 1973 [34,35]. Similarly, in 1963 and 1975 [29] approximately 14% of Dutch horses were seropositive to EAV, whereas 1.8% of German horses were seropositive in 1987, increasing to 20% in a subsequent survey in 1994 [36]. In the USA, the National Animal Health Monitoring System's Equine 1998 Study revealed that only 2.0% of unvaccinated horses in the U.S. were seropositive to EAV [37]. Similarly, resident unvaccinated California horses had a seroprevalence to EAV of only 1.9%, whereas 18.6% of horses imported into California, most commonly European Warmbloods, were seropositive [38].

The seroprevalence of EAV infection varies not only among countries, but also among breeds, for example, Standardbred and Thoroughbred horses in the USA [39,40]. In that regard, infection is endemic among Standardbred but not Thoroughbred horses in the USA, with 77.5–84.3% of all Standardbreds, but only 0–5.4% of Thoroughbreds being seropositive [15,40,41]. The seroprevalence of EAV infection in Warmblood stallions is also very high in a number of European countries, e.g., 55–93% of Austrian Warmblood stallions were positive for antibodies to EAV [42,43]. Although breed-specific differences might reflect inherent genetic differences that confer resistance to infection, they are more likely reflective of different cultural and management factors within horse populations and breeds. Studies have not demonstrated any breed-specific variation in susceptibility to EAV infection or in establishment of the carrier state [44]. This was highlighted in the 2007 series of outbreaks of EVA in France [15], with a wide range of breeds affected. The number of carrier stallions likely determines the prevalence of EAV infection within individual horse breeds and populations. The carrier state that occurs in persistently infected stallions (30–70% of those exposed), constitutes the natural reservoir of EAV, with carrier stallions venereally transmitting EAV to susceptible mares by natural service or AI [15,24,39,45]. This was the case in the most recent occurrences of EVA in the USA and France [1,15]. The emergence and spread of specific variants of EAV present in the quasispecies virus population in the reproductive tract of individual carrier stallions occurs in EVA outbreaks [22,27,28], however, the mechanisms involved in selection and emergence of virulent viral variants remain unclear (Fig. 2).

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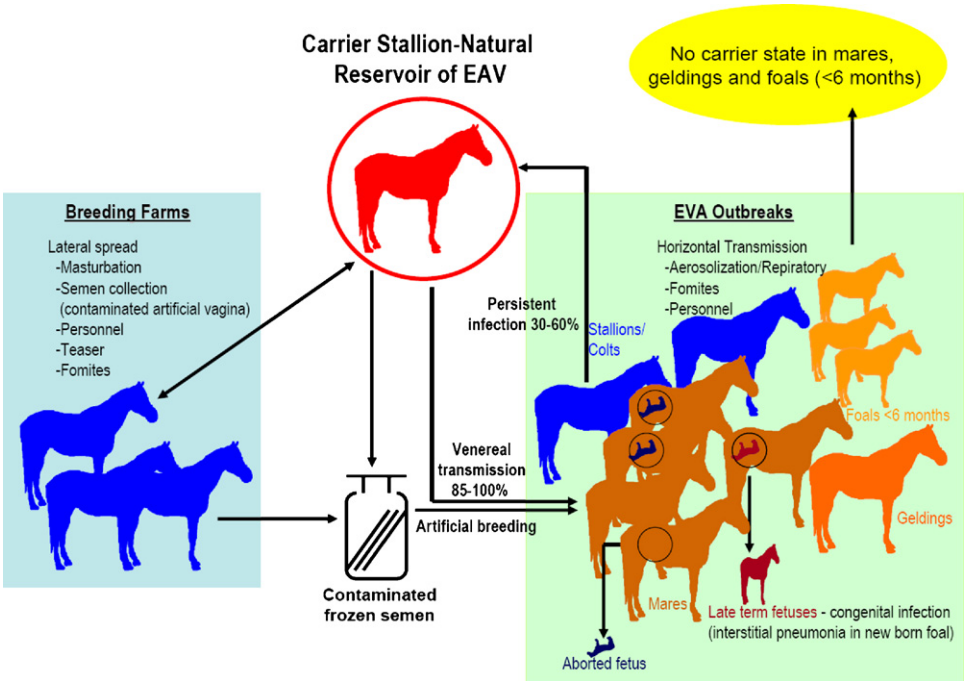

Fig. 2. Transmission of EAV between horses (adapted from [30]).

5. Pathogenesis

Within 2 d after aerosol infection, EAV is rapidly spread within the lung and bronchial lymph nodes, and then disseminated throughout the body via the circulation [46,56]. Virus can be isolated from the nasopharynx, buffy coat and serum for a variable duration following intranasal exposure (e.g., isolated from the nasopharynx and buffy coat for 2–14 d and for 2–21 d, respectively, after infection). Virus typically is isolated from serum or plasma for 7–9 d; disappearance of virus from serum coincides with the development of virus-specific neutralizing antibodies. Virus can be isolated from a wide variety of tissues and body fluids of infected horses beginning approximately 1–2 d after experimental infection by the respiratory route [46]. Apart from infrequent instances where EAV has been isolated from buffy coat cells for several months after infection, and from the reproductive tract of colts (>6 mo of age), EAV is not isolated more than 28 d after infection, except from the semen of carrier stallions [45,47,14]. While a true EAV carrier state occurs only in mature stallions, there have been a small number of cases where the virus has been recovered from the accessory sex glands for up to 180 d after experimental infection of pre-pubertal colts [48–51]. The presence of EAV in the reproductive tract of the infected mare has not been as well characterized as in the stallion. A study at the University of Kentucky documented EAV in urine, vaginal secretions, and feces [52,53], whereas studies at Oklahoma State University documented EAV in ovary, oviduct, oocyte, and uterine secretions [54].

Vascular injury in EVA likely results from direct virus-mediated injury to the endothelium and muscularis media of affected vessels. Equine arteritis virus infects and replicates in endothelial cells and causes extensive damage to the endothelium and the subjacent internal elastic lamina, and then gains access to the media of affected vessels. Vasculitis is characterized by marked fibrinoid necrosis of small muscular arteries, with extravasation of erythrocytes and proteinaceous material into the media, adventitia and perivascular tissues [17,55,56,49]. The increased vascular permeability and leukocyte infiltration resulting from generation of chemotactic factors lead to hemorrhage and edema around these vessels [57,58]. In addition to endothelial cells (EC), EAV also replicates well in macrophages (MΦ) of infected horses. Infection of cultured equine EC and MΦ leads to their activation with increased transcription of genes encoding proinflammatory mediators, including IL-1b, IL-6, IL-8, and TNF-α [59]. Furthermore, virulent and avirulent strains of EAV induced different quantities of TNF-α and other proinflammatory cytokines in both infected ECs and MΦs. These studies would indicate that cytokine

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mediators produced by ECs and MΦs have a central role in the pathogenesis of EVA.

There is evidence that abortion following experimental infection of a pregnant mare with the virulent horse adapted Bucyrus strain of EAV may be the result of a lethal fetal infection, rather than myometritis and/or placental damage impairing progesterone and pregnane synthesis leading to fetal demise/expulsion [60]. The tissues of the aborted fetus contained higher titers of virus than those of its dam, indicating that there was substantial virus replication in the fetus [60].

6. Clinical findings

There is considerable variation in clinical signs and their severity in cases of EAV infection [15,26,35,61–63]. Most infections are subclinical, especially those that occur in mares bred to many long-term carrier stallions [15,26,45]. Although there is only one serotype of EAV, as noted above, the clinical disease produced by different EAV strains can range in severity (Fig. 3) [15,60,61,64,65,66]. As with most infectious diseases, old, debilitated or immunosuppressed horses and very young foals are predisposed to more severe disease. Natural outbreaks of clinical disease are characterized by one or more of the following: abortion in pregnant mares; fulminant infection in neonates associated with severe interstitial pneumonia or enteritis; systemic illness in adult horses; and persistent infection in stallions. Cases of EVA are characterized by an incubation period of 2–14 d (6–8 d following venereal exposure) with the most consistent clinical features of EAV being pyrexia and leukopenia [15,44,66,67]. Typically, a fever (≤41 °C) may continue for 2–9 d, associated with any combination of the following: depression and anorexia; conjunctivitis and rhinitis with nasal and ocular discharge; leukopenia; peri- and supraorbital edema; dependant edema localizing in the scrotum, prepuce or mammary glands and limbs (especially hind limbs); urticaria that may be localized to sides of the neck or face, or generalized over most of the body; and abortion [15,67,68]. The clinical signs observed in natural cases of EVA vary considerably among individual horses and among outbreaks. These can be influenced by such factors as the age and physical condition of the individual, route of exposure, strain of virus and dose, and environmental conditions [15,61].

Abortion in pregnant mares is usually not preceded by premonitory signs and may occur late in the acute

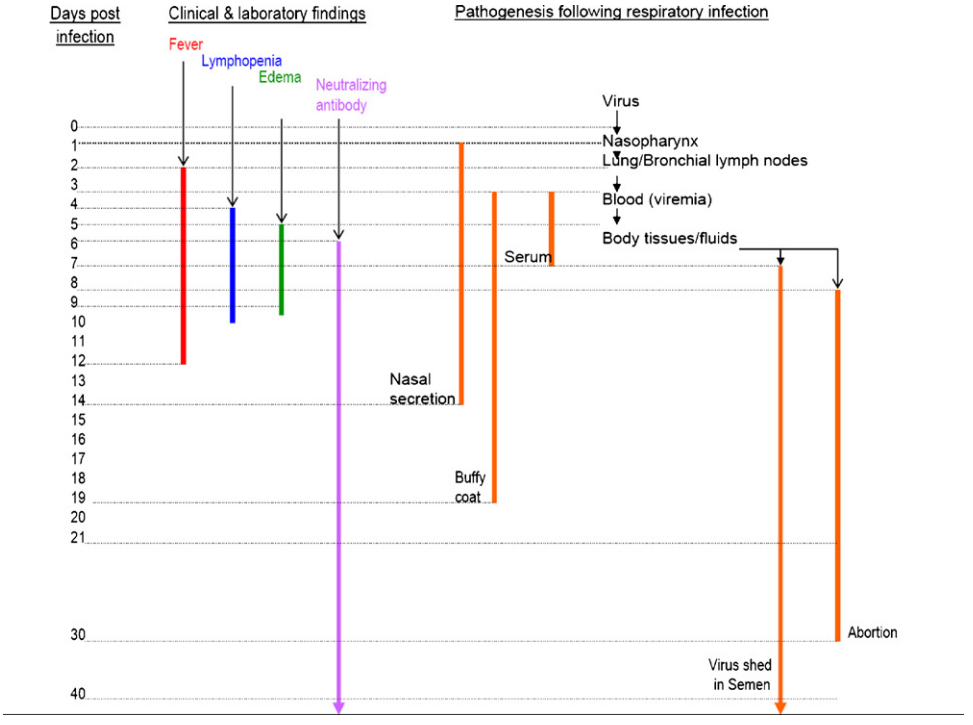

Fig. 3. Clinical and laboratory findings and sequential pathogenesis of EVA following respiratory infection. Vertical bars correspond to the chronological occurrence of the respective clinical or laboratory findings and the distribution of virus in body tissues and secretions (adapted from [30]).

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phase or early in the convalescent phase of the EAV infection [15,68]. Abortions have been documented from 3 to over 10 mo of gestation following natural or experimental infection [15,57,61]. Abortion rates in outbreaks of EVA have varied from less than 10% to as high as 50–60% [15].

During acute EVA infection, stallions may undergo a period of temporary subfertility, associated with reduced libido and decreases in sperm motility, concentration, and percentage of morphologically normal sperm. These changes can persist for up to 16 wk before returning to pre-exposure levels after experimental EAV infection of stallions [15,69]. They probably result from increased scrotal temperature, rather than any direct virus-induced pathologic effect [69], although pathology may initially be present during the acute phase of infection [49]. Semen quality is normal in persistently infected stallions, despite active shedding of the virus into the semen. Similarly, venereal infection of mares by persistently infected stallions may result in decreased fertility on the initial cycle, but it does not appear to result in subsequent fertility problems [15].

7. Diagnosis

Because clinical EVA resembles various other infectious and non-infectious diseases of horses [15], a presumptive diagnosis of EVA should not be based solely on clinical signs. The differential diagnosis of EVA includes other viral respiratory tract infections such as equine herpesviruses 1 and 4, equine influenza virus, equine rhinitis A and B viruses, equine adenovirus, and Getah virus [15]. Also to be included are equine infectious anemia, African horse sickness, purpura hemorrhagica, urticaria and toxicosis due to hoary alyssum (*Berteroa incana*) [15,70]. Histologically, EVA is characterized by a distinctive arteritis that can differentiate it from some of these other diseases, although vasculitis certainly is not pathognomonic and the severity and distribution of vasculitis varies markedly. Abortion induced by EAV can also cause a diagnostic dilemma. Differential diagnoses must include a number of non-infectious causes and infectious causes, most notably equine herpesvirus 1 (or rarely 4). Whereas in the case of EHV 1 abortion, fetuses are typically expelled without any premonitory signs, are fresh and frequently have characteristic gross and microscopic lesions, those infected with EAV are usually partially autolyzed and lack pathognomonic lesions. Laboratory diagnosis of EVA is currently based on a combination of virus isolation, viral nucleic acid or antigen detection, and serology [15].

The most appropriate specimens for virus isolation from live horses include nasopharyngeal swabs or washings, conjunctival swabs and citrated or EDTA blood samples for separation of buffy coat cells. Heparinized blood is not suitable for virus isolation because of the inhibitory effect of heparin on the isolation of EAV in cell culture [71]. Collection of semen containing the sperm rich fraction of the ejaculate is optimal for virus isolation from equine semen samples [15]. Placenta, fetal fluids, lung, spleen and lymphoid tissues should be collected for virus isolation to confirm cases of EAV-induced abortion. A wide variety of organs and lymph nodes associated with the alimentary and respiratory tracts should be collected for virus isolation in suspected cases of ''pneumo-enteric'' forms of EVA in neonates [15]. Specimens should be collected as soon as possible after the onset of clinical signs or suspected EAV infection, and nasopharyngeal and conjunctival swabs should be immediately placed in transported medium (any cell culture medium or balanced salt solution containing 2–5% fetal bovine or calf serum) and either refrigerated or, preferably, frozen at −20 °C or lower [2]. Except for blood samples, which should be refrigerated, all other specimens for virus isolation should be packed in ice and sent via overnight delivery to a laboratory approved by the USDA National Veterinary Services Laboratory, Ames, IA, USA.

Immunohistochemistry is a reliable, powerful and rapid method to diagnose EAV infection in tissues and occasionally in skin biopsies [73]. An avidin-biotin complex (ABC) immunoperoxidase staining using monoclonal antibodies to individual EAV proteins has been successfully used to detect viral antigens in formalin-fixed, paraffin-embedded tissues, as well as in frozen tissues sections [60,72–74].

Several reverse transcription, polymerase chain reaction (RT-PCR) assays, including, nested RT-PCR (RT-nPCR) and real-time RT-PCR assays have been developed for detection of the EAV nucleic acids in cell culture supernatants and clinical specimens [75–81]. Assays using RT-PCR have several potential advantages over the current virus isolation procedure. However, further standardization and validation is absolutely necessary before RT-PCR is adopted as a reliable screening assay to evaluate clinical specimens, such as those from cases of abortion and semen from horses that are suspect carriers of the virus [82,83].

For serological diagnosis of acute EAV infection, acute and convalescent sera (paired serum samples) should be collected 3–4 wk apart, and tested for either seroconversion or a four-fold or greater increase in

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serum antibody titer. The virus neutralization assay remains the gold standard for detection of serum antibodies to EAV, although several enzyme-linked immunosorbent assays (ELISAs) have been described [84–87]. None of these, however, has gained widespread acceptance.

For histopathological examination tissue samples should be fixed and saved in Modified Davidson's or 10% neutral buffered formalin.

8. The impact of EVA

A diagnosis of EVA can have profound economic consequences for both the breeding and performance sectors of the horse industry. Direct financial losses resulting from outbreaks of the disease on breeding farms include: losses due to abortion and/or disease and death in very young foals; decreased commercial value of persistently infected stallions; reduced demand to breed to carrier stallions, due to the added expense and inconvenience involved in vaccinating and isolating mares before and after breeding; and denied export markets for carrier stallions and infected semen [88]. An outbreak of EVA at a racetrack, equestrian event, or horse show can have considerable impact, due to the widespread potential for further dissemination of the virus when horses return to their farm or premises of origin [1]. This impact may include direct financial losses such as abortion, pneumonia in newborn foals, infected stallions, and disruption of training schedules, reduced competition entries, and event cancellations. The impact at the international level will affect the trade of horses and semen, due to denied export opportunities for carrier stallions and EVA-infective semen [88,89]. In fact, in the case of some countries, all categories of horses that have antibodies to the virus are affected.

9. Transmission risks

Our role as equine veterinarians is to have sufficient knowledge of this disease to interrupt the transmission cycle and establish effective programs for controlling, preventing and perhaps, eventually eliminating it. These can be summarized as follows:

Equine arteritis virus infection can be transmitted among horses in five ways:

- Respiratory: primary route of transmission in acute cases of infection, common at racetracks, shows, sales.
- Venereal: the virus shed in the semen of a carrier stallion (cooled or frozen semen can be infectious).
- Other bodily secretions: urine, feces, etc.
- In utero: the virus passes across the placenta from an acutely infected mare to her unborn foal.
- Indirect contamination: tack and/or equipment shared among horses.

There is a very real risk of EVA being transferred indirectly via personnel and fomites. Special care should be taken when handling semen in laboratories prior to insemination or preparation for shipping.

10. Control and prevention

Equine viral arteritis is a manageable disease. Effective strategies for control and prevention can be and have been designed (Fig. 4) [15,90]. These strategies include:

- Minimizing or eliminating direct or indirect contact of susceptible horses with the secretions/excretions of EVA-infected animals.
- Restricting spread of EAV in breeding populations, to prevent outbreaks of virus-related abortion or illness in young foals, and to prevent the establishment of the carrier state in stallions and post-pubertal colts.
- Establish AI industry standards!
  - ○ Determine serologic and virologic status of all stallions contributing semen that will be cool-shipped or cryopreserved and subsequently used for AI.
- Prevent the establishment of the carrier state in stallions and post-pubertal colts.
  - ○ Vaccinate colts after 6 mo and prior to 12 mo of age.
  - ○ Prevent the carrier state and the disease may ultimately be eliminated.

There is a safe and effective EVA vaccine (Arvac®, Fort Dodge Animal Health).
- This vaccine has been shown to be both safe and effective for use in stallions, non-pregnant mares, geldings, fillies, and colts.
- There is no evidence that a vaccinated stallion will develop the carrier state with vaccine virus.

Vaccination has been successfully used to help control the spread of this disease [91]. Primary vaccination affords protection against clinical disease for several years [92,93]. If initial vaccinates are exposed to field virus for the first time via venereal or aerosol transmission, they will probably have a limited re-infection cycle and be short-term shedders (up to 10

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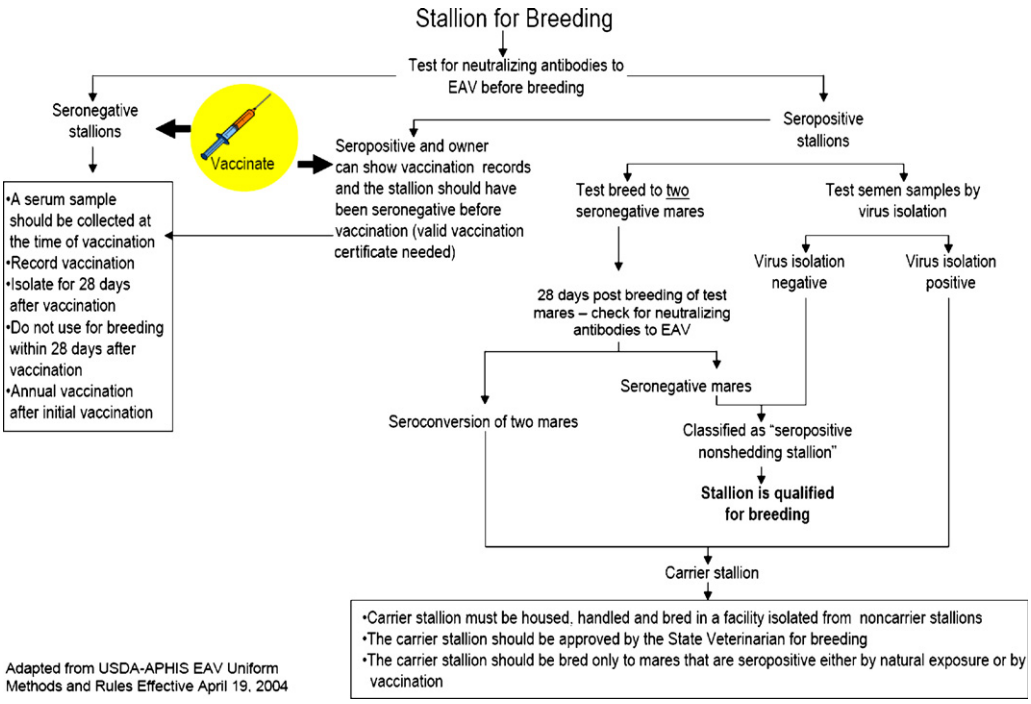

Fig. 4. Guidelines and minimum standards for prevention and control of EVA (adapted from [90]) [75].

d) of the field strain virus [52], so should be isolated from susceptible animals for 21 d. Revaccination normally results in a pronounced increase in neutralizing antibody titers and protection against the disease.

10.1. Vaccination

Vaccination strategies include:

- Vaccinate only healthy, non-stressed horses.
- If all horses are not being vaccinated at the same time on the premises, isolate those being vaccinated from those not being vaccinated (those remaining seronegative). There is a minimal potential for vaccine virus to be shed and spread to other horses.
- Vaccinate all horses according to the label instructions. (Note: Not for use in foals less than 6 wk of age, except in emergency situations when threatened by natural exposure.)

Guidelines for vaccination of horses against EAV have been prepared by the American Association of Equine Practitioners (AAEP) [94] and are presented below.

10.1.1. Stallions

Breeding stallions, *previously vaccinated*: Should receive an annual booster vaccination against EVA every 12 mo, and no earlier than 4 wk before the start of each breeding season.

Breeding stallions, *first-time vaccinates*: Prior to initial vaccination, all stallions undergo serologic testing and are confirmed negative for antibodies to EAV. Testing should be performed shortly prior to, or preferably at, the time of vaccination. Negative certification is of importance, should a vaccinated stallion be considered for export at a later date. All first-time vaccinated stallions should be isolated for 4 wk following vaccination before being used for breeding.

Teasers can play a role in the introduction and dissemination of EAV within a breeding population. Vaccination against EVA is recommended on an annual basis.

Mares to be bred to carrier stallions or to be bred with virus-infective semen should first be tested to determine their serological status for EAV antibodies.

Seronegative mares should be vaccinated against EVA and isolated from any other seronegative horses for 3 wk. The purpose of the isolation period is twofold:

(1) To enable the vaccinated mare adequate time to develop immunity against the disease before being exposed to EAV infection during breeding.
(2) To afford ample opportunity for cessation of post-vaccinal viral shedding via the respiratory tract.

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Following insemination, first-time vaccinated mares must be isolated for an additional 3-wk interval, as they are likely to experience a limited re-infection cycle with the strain of EAV present in the semen. Should such mares fail to become pregnant, they can be bred back to a carrier stallion or with infective semen without the need for revaccination or an additional 3-wk isolation period post-insemination.

Seropositive mares, having tested serologically positive for antibodies to EAV, can be bred to a carrier stallion or with infective semen for the first-time without the need for prior vaccination against EVA. After breeding, such mares should be physically separated from unvaccinated or unprotected horses for 24 h, to avoid possible risk of mechanical transmission of virus from voided semen.

Pregnant mares: The manufacturer does not recommend use of this vaccine in pregnant mares, especially in the last 2 mo of pregnancy. Under circumstances of high risk of natural exposure to infection, the vaccine has been administered to pregnant mares in order to control outbreaks of the disease. Based on early experimental studies and field experiences using this vaccine, the last 1–2 mo of pregnancy represent the time of greatest risk for a possible adverse effect on pregnancy. This was most recently illustrated in the aftermath of the 2006 multi-state occurrence of EVA, when a very limited number of abortions caused by the vaccine virus were confirmed in mares vaccinated within the final 2 mo of gestation.

Nurse mares can play a role in the introduction and spread of EAV among resident equine populations and should be vaccinated annually according to recommended protocols.

Foals: The manufacturer does not recommend use of this vaccine in foals <6 wk of age, unless under circumstances of high risk of natural exposure to infection.

Colt (male) foals: Especially in EAV endemic breeds, colt foals should be vaccinated between 6 and 12 mo of age to protect against the risk of their becoming carriers later in life. Colts should be confirmed seronegative for antibodies to EAV prior to vaccination as described above and kept isolated for 3 wk following vaccination. As foals of EAV-seropositive mares can carry colostral derived antibodies for up to 6 mo, testing and vaccination should not be performed prior to 6 mo of age.

10.1.2. Outbreak mitigation

Non-breeding population: Vaccination is an effective strategy in containing outbreaks, particularly in congregated groups of horses where isolation may be problematic. Serology testing, as described above, should be performed on intact males and females that may be intended for future breeding purposes and/or export.

10.2. Summary of prevention and control measures

1. Isolate all new arrivals for 3–4 wk.
2. If at all possible, separate pregnant mares from other horses.
   - Maintain pregnant mares in small groups according to predicted foaling dates.
3. Determine the infectivity status of all semen used for AI.
   - Prior to each breeding season, have new breeding stallions tested for evidence for EAV infection.
   - Culture semen from all non-vaccinated seropositive stallions for virus.
4. Vaccinate all non-carrier breeding stallions on an annual basis.
5. Vaccinate all immature colts after 6 mo and before 12 mo of age.

10.3. Bio-security measures in managing a persistently infected stallion

Stallion owners and stallion managers should disclose the shedding status of their stallions to mare owners, breed associations and, where required, to state authorities. Persistently infected stallions have been housed near non-infected stallions for years without transmission to non-infected stallions, and can be safely bred to adequately immunized mares or to mares that have tested serologically positive for neutralizing antibodies to EAV. This virus is readily killed under warm environmental conditions, as it is sensitive to sunlight, and low humidity. However, there has been one report of stallions becoming infected through indirect exposure to infective semen from a shedding stallion [14]. While it was unclear from this paper as to the exact mode of transmission, it was suggested that masturbatory emissions may have played a role. Normally such emissions are primarily pre-ejaculatory fluid containing little or no virus. A few stallions are occasionally observed to masturbate to ejaculation and if a stallion is sedated (e.g., with xylazine or detomadine), a chemical-induced ejaculation may occur; those emissions could contain infectious virus. To prevent aerosolization of contaminated bedding and to effect killing of the virus should any emissions occur, bedding can be dampened with a disinfectant.

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Persistently infected stallions have been safely managed with the following guidelines:

- Physically isolate any EAV-carrier stallions.
- Observe strict precautions when breeding carrier stallions or collecting infectious semen, to avoid the risk of inadvertently transferring infection via indirect contact.
- Limit breeding carrier stallions to vaccinated mares or mares with antibodies to EAV following natural exposure.
  - ○ Mares with natural titers only need to be isolated for 24–48 h after breeding, due to normal voiding of semen-associated virus.
- Vaccinate mares with negative titers at least 4 wk prior to breeding to a known carrier stallion or by AI with infected semen.
- Isolate initial vaccinated mares for 3 wk weeks post-breeding from all horses, except those known to have EAV-positive titers.
  - ○ It is especially important that these mares do not have contact with pregnant mares by any route, aerosol, respiratory and/or indirect contact, because they may shed field virus during this time.
- Isolate for 24–48 h afterwards for all subsequent breeding cycles, as with mares with antibodies to EAV following natural exposure.

11. Conclusions

Effective strategies for the control and prevention of EVA must be driven by the equine industry and facilitated by the veterinary profession. It is imperative that the equine AI industry establish standards. The primary objective is to restrict the spread of EAV in breeding populations, detect and appropriately handle infective semen, thereby preventing outbreaks of virus-related abortion, illness in young foals, and development of the carrier state in stallions and post-pubertal colts. This can be facilitated by determining the serologic and virologic status of all stallions contributing both cool-shipped and cryopreserved semen for AI, and through the prevention of the carrier state in stallions and post-pubertal colts by vaccinating colts between 6 and 12 mo of age.

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