Equine Immunology and Vaccine Strategies

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Equine immunology is an expansive field of study. Vaccines are designed to induce antigen specific immunity that provides host protection at the time of pathogen challenge. Although a great deal of knowledge exists in this field, there are areas that require further investigation. Many vaccines exist that provide strong protection while some vaccines are unsuccessful for provision of protection in all individuals or provide only short term immunity. Additional work is required to better understand vaccinology for specific diseases such as equine herpes myelitis that will eventually lead to protection from this serious infectious disease. Author's address: Kansas State University, 1800 Denison Ave., Manhattan, KS 66506; e-mail: edavis@vet.k-state.edu.

1. Introduction

The primary goal of vaccination is the induction of long-lasting and protective immunity. During the initial stages of pathogen challenge, innate immune responses are induced that may provide nonspecific clearance of the challenge. However, in addition to providing immediate protection, innate immune mechanisms also initiate adaptive immunity in the event that memory responses are required for long-term immune protection. The fundamental goals of a successful vaccine program are to safely induce immunity that provides antigen specific protection that is efficacious and long lasting. Although vaccination provides an immunologic advantage at the time of challenge, vaccine protocols should be appropriately designed in combination with effective environmental control strategies that include mosquito control programs.

2. Innate Immunity

Effective immunity requires interplay between host immune mechanisms and pathogen challenge. The initial element of host protection from such a challenge involves innate immune mechanisms. Disruption or penetration of superficial epithelium and/or mucosal barriers will result in pathogen exposure to host immunity. If immune clearance is ineffective, the potential for disease development exists. Beneath the superficial epithelium lies components of the innate immune system, which provides the host with a well-orchestrated collection of immune mechanisms that will target non-self constituents that are typically encountered during early and acute stages of pathogen challenge. If the challenge is low-grade, innate mechanisms may completely eliminate the challenge. Among the constituents of innate immunity are leukocytes that express surface proteins capable of directly signaling additional cells or secreting protein factors that will arm the host to be protected during the course of pathogen interaction.

The presence of invading pathogen is initially detected by sentinel cells that include macrophages, dendritic cells (DCs), and mast cells. These sentinel antigen presenting cells are highly capable of recognizing invading pathogen due to the presence of surface protein receptors. These receptors bind with pathogen via their pathogen associated molec-

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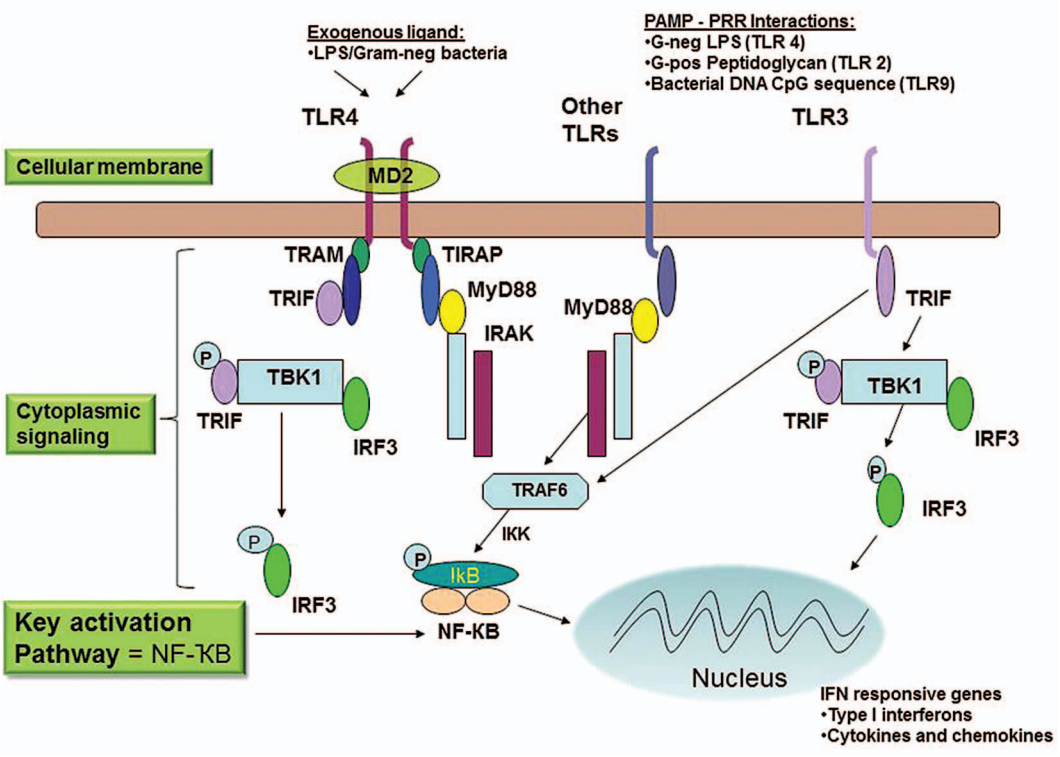

Fig. 1. Pathogen associated molecular patterns such as LPS interact with host-expressed pattern recognition receptors, which can initiate cellular signaling that results in inflammatory mediator expression.

ular patterns (PAMPs). Pathogen associated molecular patterns are expressed by a variety of pathogens such as bacteria, fungi, and viruses. Since pathogens mutate at a rapid rate, the receptors that can identify invading pathogens do not specifically identify individual microbes but rather can identify classes of pathogens that result in a global host response. Receptors on sentinel cells are referred to as pattern recognition receptors (PRRs), which may be classified into four separate categories. Pattern recognition receptors may be secreted, free receptors located in the extracellular space and in general circulation; PRRs may be membrane bound and phagocytic in function; PRRs may also be membrane bound and responsible for cellular signaling; or they may be cytoplasmic in their location. A primary example of host PRR includes the toll like receptors. Examples of PAMPs include Gram negative lipopolysaccharide (LPS), Gram positive peptidoglycan, and acid fast bacterial glycolipids. These molecules are unique to classes of microbial pathogens and are not expressed by mammalian species, which therefore provides a mechanism for host identification of pathogen challenge. Binding of PAMP with PRR results in cellular activation to trigger inflammation and other innate immune pathways (Fig. 1).

3. Adaptive Immunity

Immunologic members of the adaptive (memory) immune response include antigen presenting cells, lymphocytes, and cytokine mediators. Collectively, these constituents provide effective clearance of pathogen challenge conferring immunologic specificity and memory. Antigen presenting cells include dendritic cells, macrophages, and B lymphocytes. Dendritic cells are the most efficient antigen presenting cells, as they are the only cells that can present antigen and effectively activate naïve T lymphocytes (Fig. 2). Immature DCs are located throughout the host, and they are specifically designed to capture foreign antigen. Once stimulated by foreign antigen, DCs mature and become highly effective with regard to antigen presentation to host T cells. In order to effectively express antigen, DCs must express surface major histocompatibility molecules (MHC) class II molecules, which are essential for propagation of this process.

Dendritic cells ingest foreign antigens, process antigen in an efficient manner, and present peptides within MHC II to T cells, via the T cell receptor. Macrophages are an additional antigen presenting cell, but because they have the capacity to destroy ingested material, they are less efficient with regard to antigen presentation when compared with DCs. B lymphocytes also have antigen presenting capabilities and are highly effective during a secondary immune response.

Dendritic cells are divided into two categories, classified as myeloid and plasmacytoid. Plasmacytoid dendritic cells are particularly important for immunity and are major producers of type I inter-

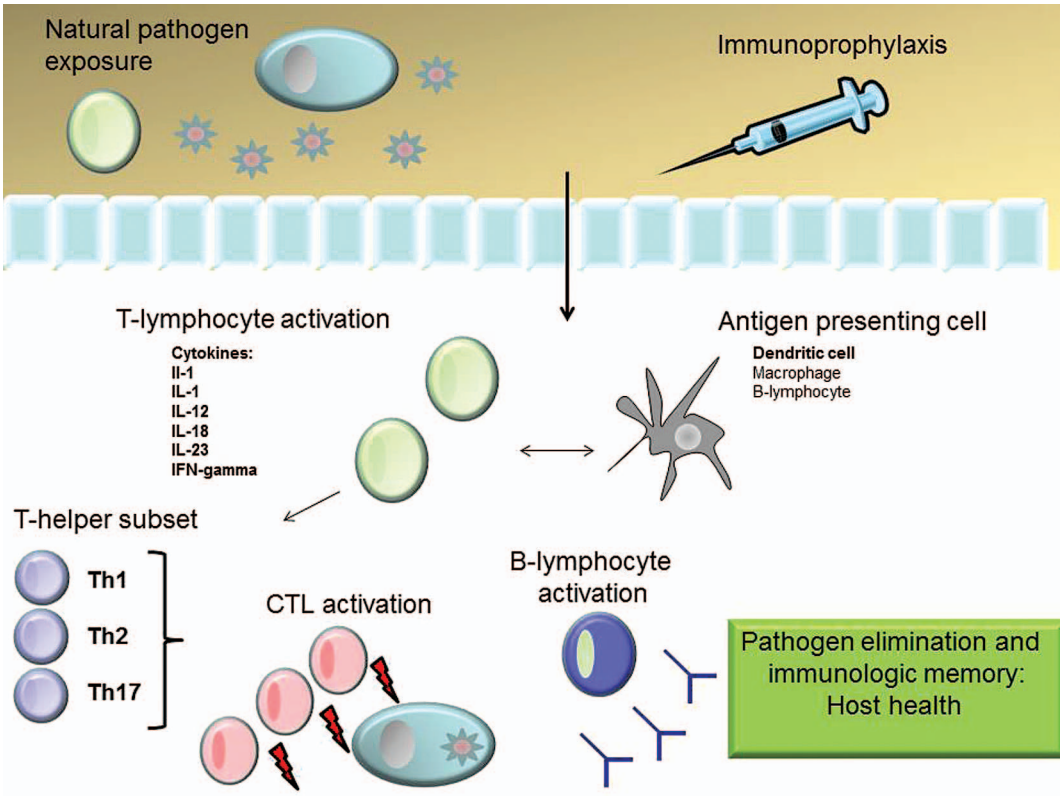

Fig. 2. Adaptive immunity involves the interaction of antigen presenting cells with T-lymphocytes in an antigen specific manner. This interaction results in clonal expansion of antigen specific lymphocyte populations capable of orchestrating pathogen clearance mechanisms.

ferons, such as interferon alpha and beta, which demonstrate pronounced antiviral properties.

Lymphocytes are found within lymphoid organs, in circulation, and scattered under mucosal surfaces. Although they morphologically appear similar, they are characterized by their cell surface molecules and their behavior. The pattern of cell surface molecules defines their specific immunophenotype. Through the process of immunophenotyping, it is possible to identify many lymphocyte subpopulations.

Helper T cells possess antigen receptors (TCRs) that consist of two peptide chains. These receptors have an antigen binding groove that can bind to antigenic peptides linked to major histocompatibility complex molecules on antigen presenting cells. The antigen binding chains of the TCR are linked to a complex signal transducing component called CD3. Each TCR is associated with either CD4 or CD8. CD4 binds to MHC class II molecules on the antigen presenting cell. CD8 binds to MHC class I molecules expressed on all nucleated cells. In order to respond to antigens, T cells must bind to antigenic peptides within the MHC protein cleft. In addition, they must also receive co-stimulation from cytokines and other co-stimulatory molecules. The signals from an APC to a T cell are communicated through an immunological synapse. There are three major types of helper T cell subpopulations (Fig. 2). Th1 cells are stimulated by interleukin 12 and secrete interleukin (IL)-2 and interferon gamma. This response classically leads to a cell-mediated immune response. Th2 cells are stimulated by IL-1 and secrete IL-1, IL-13, and IL-10. This signaling pathway generally leads to antibody production. Th17 cell development is stimulated by IL-6, transforming growth factor beta and IL-23. This cell population secretes IL-17 and promotes neutrophilic inflammation. Cytotoxic T lymphocytes are activated CD8+ expressing T lymphocytes that are essential for effective clearance of virally infected or altered self cells. Cytotoxic T cells are an important component of cell-mediated immunity.

The requirements for generating protective immunity vary with the nature of the infecting organism. Many effective vaccines work by inducing antibodies targeted toward specific pathogens. For many pathogens, including extracellular organisms, antibodies can provide protective immunity. This is not the case for all pathogens, however, such as intracellular viruses, which may require additional cell-mediated immunity that is provided by activated CD8+ T lymphocytes.

Effective protective immunity against microorganisms requires the presence of preexisting antibody at the time of infection. Antibody proteins

will aid in preventing damage from the presence of the organism or prevent infection altogether. An example of protection involves tetanus vaccination, which provides antibodies that bind even low levels of exotoxin liberated at that time of challenge. An added mechanism by which antibody proteins provide protection at the time of challenge includes the production of neutralizing antibodies. Neutralizing antibodies are capable of preventing infection by viral pathogens.

4. Host Specific Factors That Impact Immunity

Additional factors for consideration include host immunity at the time of vaccination. Horses that have undergone a stressful event such as long distance transport, exertion,[1] or have received immunosuppressive therapy[2] should not be vaccinated during times of immune suppression. It should be noted that vaccine formulation should be considered when characterizing the impact of physiologic stress on the equine patient. For instance, although exertional exercise may impede an optimal immune response, mucosal immunity appears effective in response to intranasal influenza vaccination in ponies despite high intensity exercise.[3] Specific therapeutic medications that should be taken into consideration at the time of vaccination include both corticosteroids and non-steroidal anti-inflammatory (NSAID) therapy. Previous work has demonstrated that routine doses of immunosuppressive corticosteroid therapy results in immunosuppression when used at the time of vaccine administration resulting in suppressed IgG expression.[4] Even though immunosuppressed horses are capable of producing antibody in response to vaccination, the levels of protective IgG antibody are markedly reduced when compared to IgG production in healthy horses that had not received immunosuppressive corticosteroid therapy.[4] Therefore, corticosteroid therapy is not recommended at the time of vaccination in horses. More recent data has demonstrated that the administration of an NSAID at the time of vaccination and continued for a total of 3 days can attenuate cellular and antibody responses.[5] Collectively, administration of medications that have the intended purpose of attenuation of inflammation should be used with caution if used at the time of vaccination.

Attention to health programs for young foals should be specifically designed for at risk individuals. Foals that ingest adequate colostrum will be protected from a variety of pathogen challenges during the first four to six months of life. From an immunologic standpoint, the optimal time to initiate a preventative health vaccine program is when maternal antibodies have waned, likely as the foal approaches 1 year of age.[6] From a practical standpoint, however, this is not possible, so the goal of management is to wait until maternal antibodies have fallen to a sufficient level that endogenous immunity is capable of responding with a pronounced memory response that will provide specific and long lasting protection.

We recently evaluated healthy foals that received adequate colostrum for their ability to respond to vaccination beginning at 3 months of age.[7] Measures of immunity were characterized by IgG antibody expression and CD4+ and CD8+ cellular cytokine production. When compared with foals that were initially vaccinated at 6 months of age, the foals vaccinated at 3 months of age demonstrated a similar immune profile for cellular cytokine expression including IL-4 and interferon gamma. When immune responses were examined at 11.5 months of age, similar cellular expression of interferon gamma was observed in CD4+ and CD8+ lymphocytes in response to Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), West Nile virus (WNV), influenza, and equine herpesvirus (EHV-1/4) indicating that the foals vaccinated at 3 months of age responded in a similar manner to foals vaccinated at 6 months of age ($P < 0.05$). In addition, the foals vaccinated at 3 months of age demonstrated a greater than four-fold increase in IgG expression when receiving a booster vaccine against influenza, EHV-1/4, and tetanus administered at 11 months of age. These data provide evidence that when healthy foals are initially vaccinated at 3 months of age, they are capable of demonstrating an antigen specific immune response.

Age-associated changes in immunity have been investigated in a variety of mammalian species.[8–10] Collectively, these investigations have demonstrated that there is an overall age-associated reduction in circulating T lymphocytes. Similarly, the concentration of circulating lymphocytes is reduced in aged horses.[11–13] Additional age-related changes involve lymphocyte subset concentrations. Consistent with a reduction in total lymphocyte numbers, there is a reduction in the total number of CD4+, CD8+, and B lymphocytes in circulation. However, older horses display an increased percentage of CD4+ lymphocytes.[14] Subsequently, the CD4:CD8 ratio is altered in older horses resulting in an apparent proinflammatory immune bias similar to what has been reported to occur in older people.[10]

An important characterization of lymphocyte function is the assessment of cellular proliferation induced by cytokine, mitogen, or antigen specific response in vitro. Similar to elderly humans, an impairment of lymphocyte proliferation has been observed in horses.[14,15] Regardless of the methods used to enhance this response, such as with IL-2 supplementation or changes in CD25 (IL-2 receptor) expression, cellular proliferation is not restored to adult-age responses. The conclusion from this finding is that similar to geriatric humans, horses have reduced T lymphocyte proliferation that may contribute to impaired immune function, in particular, with regard to antigen specific responses at the time of vaccination.

Since the number of geriatric horses is increasing, there is an increased need for veterinary care of this population. Many older horses continue with competitive activities into their late 20s, which may increase their risk for exposure to infectious disease. An important management program for elderly horses is to maintain appropriate and specific vaccine protocols for this at-risk population. Although there are no current recommendations that suggest older horses require more frequent vaccination than their younger stable mates, there is evidence that they may be at greater risk for disease even in the face of an appropriate preventative health program. Specifically, Adams and colleagues have demonstrates that despite having preexisting immunity to influenza, geriatric horses may still be susceptible to viral infection.[16] Therefore, the goal for equine veterinary clinicians should be to identify the specific individual or group of individuals that require vaccination. Core vaccination for potential lethal disease and those with significant zoonotic potential should be included in the protocol to include: EEE, WEE, WNV, tetanus, and rabies. Risk based decisions should also include the age and use of the horse. Respiratory pathogens such as influenza and EHV-1/4 should be included in most vaccine programs. Horses that are at low risk of other respiratory pathogens are best managed with effective environmental and biosecurity programs that minimize the potential for disease exposure. Horses at high risk for exposure should have a program that also includes additional risk-based vaccines in an effort to minimize the risk of serious infectious disease.

AAEP Vaccine Guidelines
http://www.aaep.org/info/guidelines-48

Core vaccines

EEE/WEE/WNV
Tetanus
Rabies

Risk based vaccines

Anthrax
Botulism
Equine herpesvirus (EHV-1 and 4)
Equine viral arteritis
Equine influenza
Potomac horse fever (PHF)
Rotavirus/rotaviral diarrhea
Streptococcus equi subsp. *equi* (Strangles)

Young Horse Vaccination

- Use the AAEP core vaccine protocol beginning at 4 to 6 months of age.
- Use an initial three-dose series to ensure an effective memory (secondary) immune response has been induced. This is particularly true for inactivated vaccines.
  - Administer the first two doses approximately 30 days apart.
  - Administer the third dose approximately 60 to 90 days later.
  - If the series was initiated early in the year, administer a booster vaccine at approximately 12 months of age followed by product appropriate booster vaccines.
- If the risk for disease is high, such as EEE or WNV, consider starting the series at 3 months of age to complete a three-dose series by the time infectious disease challenge is present.
  - Administer booster vaccine at 11 to 12 months of age to substantially enhance and maintain the memory immune responsiveness.

Mature Horse Vaccination

- Use AAEP core vaccine protocols for EEE, WEE, WNV, tetanus and rabies vaccination.
- If not previously vaccinated administer a second booster vaccine approximately 30 days later.
- Administer risk-based vaccines as indicated based on use and potential for pathogen exposure; consider geography and horse population for such decisions.

Elderly Horse Vaccination

- Use appropriate biosecurity protocols during infectious disease outbreaks.
- Use AAEP core vaccine protocols for EEE, WEE, WNV, tetanus, and rabies vaccination.
- Administer risk-based vaccines as indicated based on use and potential for pathogen exposure; consider geography and horse population for such decisions.
- Maintain effective booster protocols in the elderly horse population to maintain a sufficient memory immune response.
- Recognize that lymphocyte populations are reduced and altered in their expression in elderly horses; biosecurity protocols should be carefully implemented to aid in protection of this population from infectious disease.
- Identify and manage geriatric horses in an ideal preventative and health maintenance program.
  - Pituitary pars intermedia dysfunction (PPID) affected horses may require pergolide therapy to maximize overall health that includes immune function.

5. Pathogens that Provide Additional Preventative Health Challenges

Streptococcus equi subspecies *equi* (Strangles)

Vaccination for the protection of strangles is recommended in high risk individuals, such as those in-

troduced to a farm with endemic disease or a recent outbreak. Two types of *Streptococcus equi* subsp. *equi* vaccines are currently commercially available in the United States: an inactivated subunit vaccine administered intramuscularly and a modified live vaccine (MLV) administered by the intranasal route. Specific information regarding disease pathogenesis and control of an outbreak were reported in the 2005 American College of Veterinary Internal Medicine (ACVIM) consensus statement: http://onlinelibrary.wiley.com/doi/10.1111/j.1939–1676.2005.tb02671.x/pdf.

Although vaccination appears to reduce morbidity, vaccination is not without risk for adverse event. For this reason, when determining the indications to vaccinate against strangles, consideration should be made to vaccinate horses specifically at high risk for exposure to this bacterium. A potential adverse event with use of the MLV product is for inadvertent localized abscess formation. If administering concurrently with other vaccines, care should be implemented to complete administration of all other vaccines and once complete then prepare and handle the modified live product. Using such precautions will aid in avoiding inadvertent intramuscular injection of live bacterial organisms. An additional potential complication associated with use of any strangles vaccine is the development of purpura hemorrhagica, a generalized immune-mediated vasculitis that can occur following recovery from strangles infection or following vaccination, particularly if vaccination is employed during a course of disease outbreak. For these reasons, it is important for the equine clinician to carefully consider vaccine risks and consult the ACVIM 2005 consensus statement to ensure the risk for adverse event is minimized.

Horses not previously vaccinated against strangles that are receiving the MLV should receive an initial intranasal series of two vaccines approximately 3 to 4 weeks apart. Booster vaccination is recommended at 6 to 12 month intervals based on risk assessment and manufacturer instructions. Adults receiving the inactivated vaccine should be vaccinated with an initial intramuscular series of two vaccines with an approximate 4 week interval and a subsequent booster vaccine at 6 to 12 month intervals based on risk assessment and manufacturer instructions. Foals in high-risk situations should be vaccinated with the inactivated vaccine beginning at 4 to 6 months of age with an initial series of three vaccines administered at 4 to 6 week intervals. Booster vaccines should be administered at 6 to 12 month intervals. The modified live intranasal vaccine has been utilized in foals and may be administered with an initial two-dose series, starting at 6 to 9 months of age with a 3 week interval between doses. Recent evidence has suggested that the risk of adverse events associated with vaccine administration may be increased when the MLV is administered to foals less than a year of life.[17] In this reported investigation, six-month-old pony foals were vaccinated and developed clinical signs of strangles (4/4); in one foal, disease progression occurred, which resulted in mesenteric abscess formation that was diagnosed at post mortem examination, while the other three recovered without clinical intervention. When administering this vaccine in horses <1 year of age, monitoring for adverse event should be implemented.

Strangles Vaccination Considerations

- Subunit M protein vaccines.
- Modified live intranasal vaccine.
- Vaccinate in high-risk situations according to AAEP risk-based vaccine recommendations.
- Previously infected and recovered horses should be carefully considered for vaccination based on the ACVIM *Strep equi* consensus statement.
- Risk for vaccine complication and development of immune-mediated disease is increased if vaccine is administered in a disease outbreak situation.
- The risk of vaccine-associated adverse events is increased when the MLV product is administered to young foals.

West Nile Virus

Protection against WNV-induced encephalomyelitis, similar to other vector-mediated encephalitides requires strict attention to control of insects as well as an effective vaccination program. Four vaccines are currently USDA licensed for use in horses to aid in the protection against WNV. Two inactivated vaccines, a nonreplicating canary pox-vectored vaccine and an inactivated flavivirus chimeric vaccine.

Administration of inactivated WNV vaccines, consistent with label instructions, involves administration of an intramuscular dose at 3 to 6 weeks apart followed by a 12-month revaccination interval. Previous evidence has demonstrated that the clinical benefit from this class of vaccine is directly associated with protection from disease.[18] Immunologic efficacy has also been demonstrated through induction of cell-mediated immunity and antigen specific Ig subclass antibody expression.[19]

The inactivated flavivirus chimera vaccine provides host protection by expressing WNV antigens that induce protective immunity. The vaccine antigens are expressed by a yellow fever vector that safely and effectively provides antigen exposure to the host. Label instructions include a primary series of two doses administered approximately 3 to 4 weeks apart followed by an annual booster. This vaccine is approved for the aid in reduction of disease, encephalitis, and viremia.

The recombinant canary pox-vectored vaccine protective antigens are expressed in a canary pox vector, which does not replicate in the horse and contains an adjuvant. Label recommendations include intramuscular injection with a primary series

including a 4- to 6-week interval followed by a 12-month revaccination period.

An adult horse that has previously been vaccinated for WNV should receive an annual vaccine in the spring, prior to the onset of the mosquito vector season. An adult horse that is unvaccinated for WNV (or unknown vaccine status) should receive a primary series of two vaccines administered with a 3- to 6-week interval followed by annual revaccination. It should be noted that although there is limited data evaluating the safety[20] and efficacy of vaccinating pregnant broodmares for WNV, due to the inherent risk of disease it is commonplace for veterinarians to vaccinate pregnant mares. In order to induce an optimal and effective immune response, a primary vaccine series should be completed before breeding whenever possible. A booster vaccine is recommended approximately 4 to 6 weeks before expected foaling to provide WNV-specific IgG among colostral constituents.

A previous investigation demonstrated that foals, which received colostrum from WNV vaccinated mares, were capable of producing WNV specific antibodies when vaccinated at 180 days of age.[21] Current recommendations include a three-dose series for WNV vaccination to begin at 4 to 6 months of age. Booster vaccination should be administered 4 to 6 weeks after the initial vaccine with a third vaccine administered at approximately 10 to 12 months of age, prior to the onset of vector insect season the following spring. Foals that are delivered by unvaccinated mares (or those with an unknown vaccine status) should receive WNV vaccines at 3 to 4 months of age with the goal to complete the three vaccine series prior to the peak mosquito season. The duration between the first and second dose should be approximately 30 days with approximately 60 days between the second and third dose. If vaccination is taking place during the mosquito vector season, the entire protocol should be completed closer to an 8 week interval to provide optimal immunity at the time of peak mosquito season.

A recent investigation evaluated the humoral immune response to WNV vaccination with regard to serum neutralizing (SN) antibody titers generated when WNV vaccines administered as a monovalent injection or as a component of a multivalent WNV containing vaccine.[22] Vaccines that were evaluated included three commercially available WNV containing multivalent vaccines compared with their counterpart multivalent vaccines without WNV. Horses receiving a non-WNV containing multivalent vaccine were given a second injection containing the manufacturer's monovalent WNV vaccine at the same time as the multivalent product so that all groups received the same vaccine factions (EEE, WEE, tetanus, influenza, EHV, and WNV). This investigation revealed that when WNV was a component of a multivalent vaccine, WNV SN, titers were significantly diminished compared to titers generated when a monovalent WNV vaccine was administered ($P < 0.05$). These results were observed following both initial and booster vaccinations and were consistent across all three vaccine manufacturers. Many questions remain regarding all factors that contribute to a diminished antibody responses from the multivalent products, such as total antigen concentration for each vaccine and what influence other antigens such as EEE and tetanus have on immune responsiveness following vaccination. Overall, if an ideal and maximal antibody response is the goal, such as with an elderly horse or one that will encounter high risk conditions, results from this study clearly reveal that monovalent WNV vaccine will induce a stronger serologic response compared with a multivalent vaccine that contains WNV.

West Nile Virus Vaccination

- Four USDA licensed vaccines available
  - Inactivated (two manufacturers)
    - — Two- or three-dose initial series, depending on age at initial vaccination; Annual booster
  - Canarypox vectored vaccine
    - — Two- or three-dose initial series, depending on age at initial vaccination
    - — Annual booster
  - Inactivated flavivirus chimera vaccine, yellow fever vectored
    - — Two- or three-dose initial series, depending on age at initial vaccination
    - — Annual booster

Equine Herpesvirus

Equine herpesvirus-1 is associated with respiratory disease, abortion, and neurologic disease. EHV-4 is most commonly associated with respiratory disease. All horses on breeding, boarding, and training farms should be routinely vaccinated for rhinopneumonitis (respiratory form of EHV-1/4). Current commercial products include several inactivated vaccines and one modified live vaccine. Vaccination is recommended for use in pregnant mares to aid in the prevention of abortigenic EHV-1 and prevention of EHV-1/4 induced respiratory disease (rhinopneumonitis) in foals, weanlings, yearlings, young performance, and show horses that are included in the high risk group for viral exposure. There are two high antigen load inactivated vaccines labeled for protection from abortion in pregnant mares. None of the currently available vaccines has a label claim for prevention against equine herpes myelitis.

There are no vaccines that have proven efficacy for protection against the neurologic form of EHV-1 (aka, equine herpes myelitis, EHM). A comprehensive approach to manage a potential outbreak situation should be implemented when risk for outbreak exists. Outlines and recommendations have recently been reported in the 2009 ACVIM consen-

sus statement on the topic of EHV-1.[23] Current recommendations involve using appropriate biosecurity measures to minimize the potential for viral exposure. Several inactivated vaccines are commercially available, including those licensed for protection against respiratory disease alone, characterized by vaccines that contain a low antigen load and two that are licensed to provide protection against both respiratory disease and abortion, which contain a high-antigen load. Efficacy of the inactivated low-antigen load respiratory vaccine is variable, with some vaccines outperforming others. Performance of the inactivated high antigen load respiratory abortion vaccines is superior resulting in higher antibody responses and some evidence of cellular responses to vaccination. This factor may provide good reason to select the high antigen load respiratory/abortion vaccines when the slightly higher cost is not a decision factor. The most recent evidence to support the use of high antigen load vaccination for the prevention of equine herpes myelitis was reported by Maxwell and colleagues in an challenge study that aimed to determine whether an individual three-dose series of a high-antigen load vaccine[a] was effective for protecting against EHM in an established model setting.[24,25] Parameters of interest included ataxia scores, rectal temperature, and clinical scores. Control horses demonstrated more severe neurologic signs with 83% (5/6) horses affected compared to 16% (1/6) in the vaccinated group that had a minimum of two grade change in ataxia. Vaccinates had lower temperatures at times of biphasic fever spikes, significantly lower viremia and an overall clinical score 20% lower than control horses. Overall, vaccination with a high antigen load EHV-1 vaccine decreased clinical signs of infection following challenge with the neuropathic strain of EHV-1. While this investigation was a pilot study and the differences between treatment and control horses were not statistically significant, the results support subsequent investigation to establish the protective effects of vaccination in the prevention of EHM using a more robust study design.

Effective vaccination against EHV-1 should include an initial three-dose series, using an approximate 4 to 6 week interval between doses. When administering the initial series in foals, vaccination should be initiated at 4 to 6 months of age with a 4 to 6 week interval between the first two doses and the third dose being given at 10 to 12 months of age. Booster vaccines are recommended to maintain adequate immunity at approximately 6 month intervals. Pregnant broodmares should be vaccinated at 5, 7, and 9 months of gestation with a high antigen product labeled to protect against abortion. Many equine clinicians in high-risk breeding settings initiate the vaccination series at the third month of gestation. Effective colostral antibody protection is optimally induced with an inactivated EHV-1/4 vaccine booster administered 4 to 6 weeks before expected foaling. Horses maintained on breeding farms such as barren mares, stallions, and teaser stallions should be vaccinated at the beginning of the breeding season with 6 month booster vaccines administered. Adult horses should be vaccinated every 6 to 12 months, young horses (high risk, travel, commingling) will be optimally protected with booster vaccines every 6 months.

Equine Herpesvirus Vaccination Considerations

- Rhinopneumonitis (EHV-1/4) among young horses, performance horses, non-pregnant mares, initial series of three vaccines with boosters administered at 6-month intervals or according to risk of exposure.
  - Young horses <5 years of age.
  - Horses traveling to competitions and in contact with groups of horses.
  - Horses on breeding farms in contact with broodmares.
- Vaccine programs should be aimed at reducing the frequency of EHV associated disease despite recognizing that the majority of the equine population is latently infected with equine herpesviruses.
- Specific EHV-1 vaccine considerations for broodmares.
  - EHV-1 high antigen load vaccine labeled to prevent abortion administered at 3, 5, 7, 9 months of gestation.
  - EHV-1/4 rhinopneumonitis vaccine 4 to 6 weeks prior to expected foaling to enhance colostral antibody production
- No USDA licensed vaccine has a label for the prevention of equine herpes myelitis.
  - High antigen load vaccines and the MLV vaccine may induce a stronger serologic and cell mediated immunity (CMI) response that may provide the equine host with an immunologic advantage if the highly pathogenic EHV-1 is encountered.

Additional risk based vaccines:

Anthrax

Anthrax is a serious and rapidly fatal bacterial disease that has specific geographic regions associated with disease outbreak. Infections develop secondary to ingestion, inhalation, or wound contamination where soil contamination has occurred. It is important to recognize that infection is restricted to areas where alkaline soils exist, which favors bacterial survival. One USDA licensed vaccine is available for use in a variety of livestock species that includes horses. The vaccine is prepared from an attenuated, encapsulated variant of *Bacillus anthracis* strain B. Initial vaccination should include a series of two vaccines administered by the subcutaneous route followed by an annual booster while the horse is maintained in at risk areas. Vaccination is not recommended in broodmares; there is no infor-

mation regarding vaccination in horses less than a year of age. Adverse reactions have been reported in young and miniature horses, and caution should be used when use is considered in such instances.

Botulism

Botulism is a serious and frequently fatal infectious disease that develops secondary to liberation of toxin from *Clostridium botulinum*, most commonly *Cl. botulinum* type B and C. Four forms of disease occur in horses: wound botulism, shaker foal syndrome, forage poisoning, and equine grass sickness. An inactivated toxoid *Cl. botulinum* type B vaccine is licensed for use in horses in the United States. The indication for vaccination is for the prevention of shaker foal syndrome in horses less than 1 year of age. Pregnant broodmares should be vaccinated in at-risk geographic regions such as central Kentucky and the mid-Atlantic region. There is no evidence for cross protection against other *Cl. botulinum* toxins; therefore, vaccination against type B will not protect against other forms of disease.

The vaccine protocol involves an initial three-dose series in pregnant broodmares with a final vaccine booster administered approximately 4 weeks before the expected foaling date. This protocol will provide the mare the opportunity to maximize colostral antibody concentration at the time of parturition. Annual boosters are recommended at time of prefoaling vaccination. Foals in high risk regions should begin a vaccine series at 2 to 3 months of age. Foals born to unvaccinated mares or foals moving to high risk geographic locations should begin the three-dose vaccine series at 2–4 weeks of age with boosters at 4 week intervals. Other horses located in high risk regions should receive an initial three-dose series with annual booster vaccination. Horses that have suffered from *Cl. botulinum* type B infection that have recovered from disease should begin a three-dose vaccine series following recovery.

Equine Influenza

Equine influenza is a common upper respiratory infection resulting from viral colonization of the upper respiratory tract. Equine influenza is classified among the orthomyxoviruses, which are single stranded RNA viruses. Influenza viruses are classified among three types depending on surface and internal protein antigens, A, B, and C, yet only type A influenza has been reported to infect horses. Major viral antigens include neuraminidase and hemagglutinin. Two type A subtypes that are known to cause disease in horses include H7N7 and H3N8. Among viruses H3N8 is considered to be of greater pathogenicity than H7N7.

Influenza is most common in horses commingled under stressful conditions such as race or show training. Infection occurs via inhalation of viral particles, which are abundant in an environment containing infected horses, resulting from severe paroxysmal coughing that occurs in affected individuals. Once inhaled, the virus infects the respiratory ciliated epithelium, leading to loss of the mucociliary escalator, which substantially diminishes pathogen and particle clearance. Therefore, viral infection predisposes affected individuals to secondary bacterial colonization.

Clinical manifestation of disease includes a short incubation period of 1 to 3 days, high fever, depression, and paroxysmal coughing, which can be severe. Nasal discharge typically begins as a serous fluid, yet with disease progression and bacterial contamination mucopurulent discharge may be observed.

There are currently three influenza vaccines available:

1) *Inactivated (intramuscular administration)*: This vaccine contains many of the A2 stains of influenza that are currently in circulation. These vaccines are recommended to include a three-dose priming series with the initial dose administered with a 4 to 6 week interval; the third vaccine should be administered 8 to 12 weeks after the second dose. These vaccines are well suited for prefoaling series, which will provide high levels of colostral immunoglobulin.
2) *Modified live cold-adapted equine influenza A/2 (intranasal administration)*: This vaccine is administered intranasally and has been shown to effectively provide rapid protection to naïve individuals.[3,26,27] The product is labeled for protection of horses 11 months of age or greater. Vaccination in horses 6 months of age and older is recognized to safely provide protection from disease. Label claims report 6 months of protection; however, it is recognized to confer protection for 12 months. Circulating immunoglobulin levels are not markedly elevated following vaccination; local mucosal immune protection is recognized to confer immunity to individuals following vaccination.
3) *Canary pox-vectored vaccine (intramuscular administration)*: This product is administered as an intramuscular injection and is safe to administer as early as 4 months of age. A strong humoral response is observed following vaccination, suggesting that this is also an appropriate product to administer during late term pregnancy to enhance colostral antibody levels.

Adult horses that have previously been immunized and are at risk for exposure can be effectively vaccinated twice annually. If risk for disease in relatively low, annual booster vaccination should be sufficient to provide protection from disease. Previously unvaccinated adult horses can effectively be vaccinated with an individual dose of the MLV intranasal vaccine and boostered at 6 month intervals. Alternatively, an initial two-dose series of the canary pox vectored vaccine or a three-dose series of

an inactivated vaccine should provide protection. When administering a three-dose series of the inactivated vaccine a period of 3 to 4 weeks between the initial two vaccines followed by a period of 3 to 6 months before the third vaccine will provide optimal protection. Subsequent revaccination should be performed at 6 to 12 month intervals, depending on the use and risk of exposure of the horse.

Horses in the first year of life and less than 11 months of age should begin receiving vaccination at approximately 6 months of age. If the MLV intranasal vaccine is selected, the initial dose should be administered at 6 to 7 months of age with a booster vaccine administered at 11 to 12 months of age. If the canary pox-vectored vaccine is administered, it should be initiated at approximately 6 to 7 months of age with a booster vaccine administered in 4 to 6 weeks. If the inactivated vaccine is selected, it should be administered in a three-dose series with the initial dose at approximately 6 months of age, the second dose approximately 4 to 6 weeks later, and a third dose 3 to 6 months later. If the foal is born to a mare that is suspected to be unvaccinated, it is still likely that some maternal antibody transfer has occurred; due to the effect of maternal antibody interference, the vaccine series should be initiated at approximately 6 months of age. Broodmares previously vaccinated should receive a booster vaccine approximately 4 to 6 weeks before expected foaling with either an inactivated product or the canary pox-vectored vaccine. If a broodmare is previously unvaccinated, a three-dose series of inactivated product should be administered with an interval of 4 to 6 weeks between the first two vaccines followed by a third dose at approximately 4 to 6 weeks before expected foaling. Alternatively, a two dose series of the canary pox-vectored vaccine can be administered so that the second dose of vaccines is administered approximately 4 to 6 weeks before the expected foaling date.

Potomac Horse Fever

Equine monocytic ehrlichiosis is caused by *Neorickettsia risticii* (formerly *Ehrlichia risticii*) and causes a seasonal course of disease in late spring through early fall. Clinical signs may include fever, diarrhea, laminitis, colic, and reduced gastrointestinal motility. In endemic regions, one component of reducing disease severity includes vaccination.

Two commercial inactivated vaccines are available for use in horses considered high risk for disease development. Pregnant mares that develop disease are at risk for abortion; neither of the commercially available vaccines claims to protect against abortion. Adult horses that have previously been vaccinated should be vaccinated prior to the peak season associated with disease, typically spring months. Booster vaccine administration is recommended at 3 to 4 month intervals in high risk situations, as the duration of immunity induced by vaccination appears to be short lived. Horses that have not been previously vaccinated should receive an initial series of two vaccines at 3 to 4 week intervals with the second dose being completed approximately 2 to 3 weeks before the anticipated peak incidence of disease. Broodmares should receive a booster vaccine approximately 4 to 6 weeks before expected foaling. If the mare has not been previously vaccinated for Potomac horse fever (PHF), she should receive a two dose series with the second dose being administered approximately 4 to 6 weeks before expected foaling. In foals, the initial dose should be given at 5 to 6 months of age with a second dose to follow in 3 to 4 weeks; a third dose should then be administered at 10 to 12 months of age. Boosters are then recommended at 3 to 6 month intervals depending on the risk of disease. Boosters should be given in May to June in endemic regions.

Rotavirus

Rotavirus is a nonenveloped RNA virus that is an important infectious cause of foal diarrhea. Although many factors play important roles for the development of diarrhea in foals, such as environmental conditions and exposure to infected individuals on high density farms, mare vaccination against rotavirus results in high antibody levels in colostrum and subsequently increased foal serum antibody concentrations. Evidence to support mare vaccination is based on field trials, which have demonstrated attenuation of rotaviral diarrhea cases.

One inactivated vaccine is commercially available, which contains group A rotavirus and is indicated for use in mares to enhance colostral immunoglobulin concentration against equine rotavirus (group A). Mares should receive a series of three doses of intramuscular vaccines at 8, 9, and 10 months of gestation, regardless of previous vaccine status. This vaccine is used frequently in regions where large populations of mares and foals are housed; in addition, mares that are being prepared to be shipped to high density areas should be vaccinated in preparation for exposure to rotaviral challenge. It is important to ensure that foals receive an adequate quantity of colostrum from mares that have been vaccinated against rotavirus. There is no evidence to suggest that newborn foals require vaccination against rotavirus. Colostral antibodies will decline at approximately 60 days of age; although there is some risk for development of rotaviral diarrhea at this age, the risk is considered low and if disease develops it is generally self-limiting.

Rattlesnake Bite Envenomation

Venomous snake bites occur in certain areas of North America. Based on risk for exposure there may be an indication for vaccination of horses in high risk areas with the *Crotalus atrox* (Western Diamondback Rattlesnake) toxoid vaccine. Currently there is one toxoid vaccine with a conditional license indicated for use in horses that are at risk for rattlesnake envenomation. The label suggests that

there may be cross protection against the Western Rattlesnake (including Prairie, Great Basin, Northern, Southern, and Pacific varieties) Sidewinder, Timber Rattlesnake, Massasauga, and Copperhead. Partial protection may be provided against the Eastern Diamondback but not the Water Moccasin (cottonmouth), Mojave Rattlesnake, or Coral Snake.

The toxoid vaccine is labeled for use in horses older than 6 months of age. The primary series includes three vaccines to be administered at monthly intervals with subsequent booster vaccination at 6 month intervals. Use of this vaccine in pregnant broodmares has not been specifically investigated, and if needed, direct consultation with the manufacturer is recommended.

6. Summary

Effective vaccine programs are best achieved by identifying AAEP established core and risk based needs. Recognition of all factors that may influence equine immunity should be carefully evaluated. An understanding of equine immunology provides the practitioner with a strong foundation for selecting specific vaccine protocols. Identification of factors that may influence immune function such as age, stress, and pharmacologic agents are important considerations when establishing the ideal vaccine protocol. Specific infectious diseases may provide unique challenges based on disease manifestation and available commercial vaccine products. Due to the expansive nature of the equine industry and the diversity of the equine population, it is imperative that equine veterinarians continue to serve as the primary source of information as horse owners and equine professionals design and implement vaccine programs.

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Conflict of Interest

The Author declares no conflicts of interest.

References and Footnote

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[a]Pneumaboart K®, Zoetis, Kalamazoo, MI 49007.