Evidence-Based Immunization in Horses

Nuria Barquero, DVM, MSc, MRCVS\textsuperscript{a},
James R. Gilkerson, BVSc, PhD\textsuperscript{b},
J. Richard Newton, BVSc, MSc, PhD, FRCVS\textsuperscript{a,*}

\textsuperscript{a}Centre for Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, United Kingdom, CB8 7UU
\textsuperscript{b}Equine Infectious Disease Laboratory, Veterinary Pre-Clinical Centre, Faculty of Veterinary Science, University of Melbourne, Victoria, 3010, Australia

Immunization, or vaccination, may be defined as the process of rendering a subject resistant to disease by administration of vaccines for prevention, amelioration, or treatment of infectious diseases. The twentieth century was a period of great leaps forward in veterinary science. At the forefront of these advances was the development of vaccines that enabled veterinarians to control and prevent diseases that had previously been devastating to the livestock industries. As had been proven in the field of human public health, vaccination campaigns were central in the control of these infectious diseases and, in many cases, were used to eradicate previously endemic and economically crippling diseases, such as foot and mouth disease and rinderpest. In the field of equine medicine, however, the economic impetus for development of efficacious vaccines was not as great as for the livestock industries. Thus, in many respects, it has lagged behind.

Evidence of vaccine efficacy is essential for practitioners when giving advice to clients about the relative merits of different vaccines or when trying to evaluate the economic benefits of instituting a vaccine program. Vaccine efficacy data are relatively readily available in the field of human health. When examining the evidence of efficacy of human vaccinations, there is not only the published data available from phase 2 and phase 3 trials but real-life postrelease field data. There are many governmental and nongovernmental public health agencies that record the details of vaccination programs in children as well as for particular global public health...
initiatives, such as the smallpox eradication program. Many childhood
diseases, such as measles, are notifiable in most developed countries, and
such data as the total number of children vaccinated, the number of re-
ported cases of disease, and the number of adverse reactions to vaccination
are generally well reported by the public health services in many countries
around the globe.

Unfortunately, in veterinary medicine in general and in equine veterinary
medicine in particular, this sort of data, which is necessary to make
informed decisions about vaccine use and effectiveness, is often not avail-
able. The most commonly available evidence that many veterinarians have
at their disposal is the registration claim of the product, and perhaps the
safety and efficacy data that were required for registration, which may or
may not have been published in a peer-reviewed journal. Double-blind
randomized controlled trials are uncommon. In many cases, there is a con-

clict between the expectations of the owner and the data at hand for the
veterinarian. The owner expects that vaccination is going to prevent the
disease in question, whereas the product may only claim to be an aid in
the management of the disease, which can lead to dissatisfaction on behalf
of the client when the disease still occurs in fully vaccinated animals. Veter-
narians need to consider the epidemiology of the disease in question, the
type of vaccine that they are administering to the animal, the immunologic
constraints of the vaccine technology, and the available evidence of efficacy
when they are evaluating which vaccine to use or whether to vaccinate at all.

The use of vaccines in controlling equine infectious diseases should be
considered as only a part of a more wide-ranging strategy in disease control.
In dealing with disease outbreaks, predetermined actions need to be taken,
including strategies for isolation of affected animals, limiting shedding of
pathogens into shared environments, heightened hygiene practices, and,
possibly, adoption of vaccination in the face of an outbreak when indicated.
Disease control at the herd level should be instigated through limiting the
contact between infected and susceptible animals. The implementation of
multiple simultaneous disease control measures, which includes the use of
vaccination, may present some difficulties with respect to evaluating the
true effectiveness of immunization in preventing, ameliorating, or treating
infectious diseases, however.

Mechanisms of action

Immune defenses include innate and adaptive responses, but only the
adaptive responses can be induced by vaccination. The immune adaptive
response is mediated by antibodies or by effecter cells, such as cytotoxic
T lymphocytes (CTLs) and T-helper (TH) lymphocytes (CD4+). There
are two subsets of TH cells: TH-1 cells, which stimulate cytotoxic and
inflammatory functions, and TH-2 cells, which stimulate antibody response.
Vaccination should induce the appropriate TH response, which is influenced by the type of antigen, the type of adjuvant, and the immunization route.

Various different technologies are used in equine vaccines at the present time. In killed vaccines, the agent is completely inactivated using heat, chemicals, or irradiation; in most cases, their efficacy requires the addition of a potent adjuvant. Vaccines in this group include inactivated whole-pathogen vaccines, which are currently the most common equine vaccines; protein subunit vaccines, which incorporate inactive pathogen proteins; and recombinant subunit vaccines, which contain synthetic antigen produced using recombinant DNA technology.

In live vaccines, the pathogen is alive but expresses attenuated pathogenicity. These vaccines generally have a longer lasting duration of immunity, but they may potentially induce disease in immunocompromised animals. Vaccines in this group include modified-live vaccines, which may be attenuated through multiple cell culture passage using variants from other species or by development of temperature-sensitive mutants, and recombinant vector vaccines, which use pathogen DNA inserted in another non-pathogenic organism to express pathogenic and immunogenic peptide epitopes. Additional technologies are being investigated for use in horses but are currently not available as commercial vaccines; these include reassortant virus vaccines and DNA vaccines.

Evaluating the evidence

As with other aspects of equine veterinary medicine, it is appropriate to assess the scientific merits of immunization objectively in its intended role of disease prevention, amelioration, or treatment. This is important because vaccine production, promotion, and administration are a financially lucrative part of the equine veterinary health care business, and it should be incumbent on the veterinary profession to ensure that it is seen to use this technology for improving the health and welfare of animals in its care rather than simply as a money-making exercise. To this end, evidence-based veterinary medicine (EBVM) provides an increasingly powerful tool with which to examine the present level of proof for endorsing beneficial consequences from vaccination of horses against infection with and disease caused by a range of specific pathogens. As in all forms of EBVM, the quality or strength of the evidence varies across a spectrum according to the type of study that is presented. At the top of a theoretic pyramid of quality of evidence sits multistudy meta-analyses, followed by systematic reviews and randomized clinical trials and then by selective reviews and nonrandomized clinical trials. Behind these higher quality studies come cohort, case-control, and cross-sectional studies in that order, and the lowest quality evidence is provided by case series and reports and, finally, expert opinion.

In applying these levels of evidence for immunization against equine infectious diseases, we are not in fact aware of any notable peer-reviewed
meta-analyses among this broad subject and reviews tend to be selective rather than truly systematic. Rather, much of the evidence relies on experimental challenge studies of varying quality as opposed to the randomized clinical field trials that are conducted in target human populations but remain rare for equine vaccines. Specific limitations of experimental studies may relate to insufficient sample size to detect a statistically meaningful effect from vaccination; absence of suitable masking (often referred to as blinding) of study investigators, thereby introducing unintentional observer bias; and poor generalizability to target populations through use of a healthy subset of age-restricted subjects that have been evaluated under optimal experimental conditions.

Another source of evidence relating to the effectiveness of equine vaccination in preventing infection and disease is based on field outbreak investigation. Failure of vaccination to prevent disease in the field may be related to significant antigenic and pathogenic differences between the infectious organisms included in the vaccine and those responsible for the infection, often referred to as strain variation. This is often in contrast to experimental challenge studies, in which vaccine strains and infecting viruses may be the same or closely related. Field-based cross-sectional surveys have also been used to evaluate the ability of vaccines to stimulate measurable immune responses previously demonstrated as being valid proxy measures of susceptibility to infection and disease.

**Equine influenza virus**

Among naive horses, equine influenza is a highly contagious respiratory disease and is characterized by pyrexia, associated depression and anorexia, a harsh dry cough, nasal discharge, and secondary bacterial respiratory infection. A novel H3N8 equine influenza A virus subtype, which first emerged in Miami, Florida in 1963, initiated a worldwide pandemic of equine respiratory disease [1–3] and was the stimulus for the development of multivalent adjuvanted influenza vaccines for horses [4–7]. This early work, based on experience from human vaccines, led to the development of the now broadly standard schedules for equine influenza vaccination. These schedules recommend that a primary course of two doses of injections be given approximately 4 to 6 weeks apart, followed by a booster vaccination 6 months after the end of the primary course and annual boosters thereafter. The same schedules are still adopted today for the product datasheet recommendations for the latest inactivated virus vaccines and are the basis for the regulatory rules for most international equine competitions.

It was recognized during several influenza outbreaks in the United Kingdom during the 1970s [8–11], and especially in the 1979 outbreak [12], that vaccinated horses generally experienced less severe disease than those that were unvaccinated. Influenza vaccination of Thoroughbred racehorses
in Great Britain became mandatory under the Jockey Club Rules of Racing at the start of the flat-racing season in March 1981 and was followed soon after in Ireland and France. Since that time, British racing has not been cancelled because of equine influenza, but there have been continued seasonal peaks of infection among unvaccinated non-Thoroughbred horses associated with increased mixing at shows in the summer months. Because of the absence of systematic, consistent, and long-term surveillance data, however, it is not possible to provide conclusive evidence to support the true impact of mandatory influenza vaccination on reducing the incidence of equine influenza virus (EIV) infection and associated disease.

With the evolution of equine H3N8 influenza A viruses attributable to antigenic drift, influenza outbreaks have periodically caused periodic disruption to the training schedules of vaccinated Thoroughbreds in individual yards or training centers in the United Kingdom, despite the use of vaccination [13–16]. The adoption of more widespread vaccination has made the diagnosis of influenza infection less straightforward, however, because clinical signs are less severe, acute blood samples already possess moderate levels of serum antibody, and the quantities of live virus retrievable from the respiratory tract are greatly reduced. The development of a sensitive and rapid ELISA for the detection of influenza nucleoprotein (NP) antigen in extracts from nasopharyngeal swabs [17,18] has greatly improved the ability to diagnose influenza in previously vaccinated horses, as was demonstrated in the 1989 United Kingdom outbreak [14].

Failure of vaccine efficacy is commonly referred to as “vaccine breakdown” and is attributed to a combination of variable vaccine potency, poor response to vaccination, and antigenic drift in EIVs [19–21]. Measurement of serum antibody by the single radial hemolysis (SRH) test has been shown to be particularly sensitive for detecting influenza infection, and SRH antibody has predictive value for vaccine-induced protection [22–24]. Although cell-mediated immunity (CMI) has been suggested to be an important component of immunity to influenza, there is little evidence that it is stimulated by inactivated vaccines [25,26], although some evidence is now emerging that novel vaccine approaches based on canarypox vectors may induce meaningful CMI [27].

Small-scale controlled experimental studies using vaccinated and nonvaccinated influenza-naive Welsh Mountain Ponies provide the main evidence for the pattern of serologic responses after administration of influenza vaccines and the protective immunity that they provide. Experimental influenza viral challenge studies have repeatedly shown a strong correlation between vaccine-induced humoral antibody levels and protective immunity against infection with antigenically similar viruses (“homologous” viruses) [28–32]. Subsequent field studies have confirmed these observations in vaccinated racehorses with natural infection [24,33]. Field studies have also confirmed the need for inclusion of antigenically relevant strains in vaccines for the strong correlation between vaccine-induced antibody and
immunity to remain valid [14,34], thereby corroborating findings from experimental strain variation challenge studies [35].

Several potentially important differences exist between experimental studies and the field situation, however, particularly in relation to interference with vaccine responses from maternally derived antibody (MDA) as well as the use of different types of vaccines administered in immunization schedules. Experimental studies, frequently conducted for licensing purposes on behalf of commercial companies, necessarily use the same vaccine (adjuvant and antigen strains) under optimal cold-chain conditions for all vaccinations, which are usually administered to recently weaned pony foals that have no serologic evidence of MDA. In contrast, the field situation may involve different vaccine types that are transported under suboptimal conditions and administered to horses from well-vaccinated dams. The effects of mixing vaccine types on serologic responses in experimental ponies have not been characterized, but observations from a field outbreak in Newmarket in 1995 raised the possibility that mixing of vaccine types played a part in the failure of vaccine efficacy [33]. It has also been proposed that persistence of passively acquired MDA might interfere with vaccine responses in young horses through neutralization of vaccine antigen [36–39].

It has long been recognized that young horses, especially groups of racehorses, are particularly susceptible to influenza-like infection [40], and it is harder to stimulate vaccine-induced immunity in these young horses than in older animals [5]. Failure of efficacy of vaccines is still most commonly reported in young racehorses, most likely for various reasons, including vaccine potency, the horse’s immunologic response, and differences between the infecting and vaccine viruses [33]. In addition, international movement of horses has been recognized as an important factor in the spread of influenza throughout the world [16,41–43].

Although a reasonably strong body of evidence exists for EIV vaccine efficacy based on experimental vaccination and challenge studies conducted under optimal conditions in previously nonvaccinated ponies, little randomized controlled evidence exists for such vaccines being effective in field conditions. A notable exception is provided by a study describing a double-blind, randomized, placebo-controlled trial of a vaccine among 462 stabled horses at a Canadian racetrack [44]. Horses vaccinated with an inactivated aluminium phosphate–adjuvanted vaccine did not differ from those that received placebo in the severity of clinical signs in the face of a natural EIA outbreak. The results of that study showed no significant decrease in the risk of developing infectious upper respiratory tract disease between vaccinated and nonvaccinated horses, although the median duration of clinical disease was 3 days shorter in vaccinated horses [44,45].

The qualitative differences between the immune responses that follow infection or vaccination with inactivated virus suggest that improvements can be made in vaccine design. Ideally, vaccines should induce broadly reactive, local and systemic, antibody and cellular immune responses; establish
memory; and consequently generate a rapid anamnestic response on field exposure to EIV. The occurrence of free and cell-associated virus is thereby reduced, and recovery is enhanced. Live-attenuated and live-vectored equine influenza vaccines that should more closely mimic natural infection are now available. A live recombinant vaccine that uses canarypox as a vector to express the haemagglutinin (HA) genes of EIVs has been available in Europe since 2003. The recombinant virus undergoes an abortive infection in mammalian cells, such that no progeny viruses are made but the expressed viral antigens are processed endogenously and presented as peptides by means of major histocompatibility complex (MHC) class I by the host cell in the same manner as occurs in natural infection but without associated infection risks. Experimental challenges with the canarypox-vectored vaccine showed that horses were protected against infection and virus shedding was markedly reduced [46]. The experiment used 15 influenza-naive Welsh Mountain Ponies randomly assigned to three groups of 5 ponies. The two vaccinated groups (one dose and two doses) had statistically significantly less severe clinical disease than the control group and did not shed as much virus. Another study used 24 ponies in an experimental challenge and also showed that the canarypox vaccine protected against development of clinical signs and viral shedding [27].

A cold-adapted, temperature-sensitive, modified-live virus equine influenza vaccine that is delivered intranasally is now licensed for use in the United States. The safety and efficacy of the vaccine have been demonstrated in experimental studies; however, the vaccine does not provide sterile immunity [47–50]. No correlation was found between the concentration of serum antibody induced by vaccination and protection against infection, although an anamnestic response was demonstrated at 7 days after infection [49]. Although there is evidence to show that primed animals develop a serologic response [50], it seems that the use of serum antibody response as a measure of live virus mucosal vaccines in naive animals is inappropriate. Although the development of reliable infectious challenge models in horses allows the experimental efficacy of all types of EIV vaccines to be consistently evaluated, the inability to measure alternative correlates of immunity for alternative vaccination strategies presents problems for the newer vaccines in extending their evaluation of effectiveness to the field situation.

Summary

The reliable experimental reproduction of EIV infection and associated disease provides strong evidence for the effectiveness of a wide range of vaccine types, including newer technologies in which good correlates of protection are not yet measurable. Although equine influenza vaccines have been available and used for several decades, convincing field-based data are extremely limited; in fact, the only truly blind randomized controlled field trial indicated that the vaccine under study was not effective.
Some care is required in interpreting experimental studies conducted under optimal conditions that use nonvaccinated control groups for comparison with vaccinated animals, because it is relatively straightforward to show apparently significant benefits from vaccination in terms of reduction of clinical signs alone. Nevertheless, it is clear from the long distance and almost global spread of equine influenza in spite of aggressive vaccination strategies that elimination of viral shedding is a more important goal, and more relevant benchmark for investigation, than is attenuation of clinical signs if future preventive strategies are to be successful.

**Equine herpesviruses 1 and 4**

Equine herpesvirus (EHV) 1 was first isolated from an aborted fetus at necropsy in 1932 in Kentucky [51]. Since then, EHV-1 has been recognized as a significant cause of equine fetal and neonatal losses in horse-breeding populations worldwide [52]. It was first thought that EHV-1 was responsible for outbreaks of rhinopneumonitis and abortion, but restriction endonuclease studies identified that the respiratory isolates were substantially different from the abortion isolates [53–55]. This recognition subsequently led to the differentiation of EHV-1 and EHV-4.

EHV-1 and EHV-4 are spread by the respiratory route, but EHV-1 rapidly progresses to establish a systemic infection by means of a lymphocyte-associated viremia, whereas EHV-4 remains primarily in the respiratory tract. Viremia is central to the pathogenesis of EHV-1 abortion and neurologic disease; viremia is the mechanism of transport of virus to the sites of secondary replication, such as the uterus and spinal cord [56]. Transportation to replication sites distant to the site of primary replication in the upper respiratory epithelium and EHV-1 abortigenic disease have been demonstrated to occur in mares with high levels of antibody [57,58]. Thus, the use of antibody alone as a marker for protection against EHV-1 systemic disease is of dubious value.

Recent seroepidemiologic studies in Australia have shown that a small proportion of foals are infected with EHV-1 early in life [59,60]; subsequent detection of EHV-1 and EHV-4 in nasal samples from foals less than 1 month old confirmed these findings [61]. The source of EHV-1 infection for these foals was likely to have been reactivation of a previously latent infection in the mare [60]. This silent cycle of EHV-1 infection commenced with mare-to-foal spread, with subsequent foal-to-foal spread in the preweaning and postweaning periods [60,62]. These data have implications in the design and implementation of vaccination programs, because these infected foals are lifelong latently infected carriers of EHV-1 that can become a source of infection for horses in the future. The age of first infection also is problematic, because some foals are infected before an age when they have a maximal immunologic response to the vaccine.
The immunopathogenesis and history of EHV-1 vaccination have been comprehensively reviewed [63]. An early strategy for controlling EHV-1 abortion by vaccination was based on the use of hamster attenuated-live virus in a “planned infection” program [64,65]. This attenuated virus was fully infective by the respiratory route and spread from vaccinates to in-contact susceptible horses [66]. The program was successful in reducing the incidence of abortion in mares on the breeding farms in Kentucky, where it was tested, as well as in reducing the frequency of rhinopneumonitis in racehorses [64]; however, abortions associated with planned infections, the short duration of immunity, and the period of quarantine that was required after treatment led to this method of vaccination being superseded [66]. Other attenuated vaccines have subsequently been used to control EHV-1 abortion. Another EHV-1 vaccine, first hamster attenuated, and then further attenuated in equine cells, was developed in Europe [67]. This vaccine was widely used in Europe and North America; however, questions of efficacy [68–70] and reports of abortion storms in vaccinated mares [70] led to the increased use of inactivated vaccines.

Inactivated EHV-1 vaccine and, more recently, a vaccine against EHV-1 and EHV-4 combined have been the most commonly and widely used EHV vaccines. Several trials of inactivated EHV-1 vaccine have demonstrated that the frequency of EHV-1 abortion in vaccinated mares was reduced [57,71] when using a chemically inactivated adjuvanted vaccine administered by intramuscular injection. Several subsequent studies have examined the efficacy of inactivated EHV-1 vaccines, with mixed results. One large-scale field trial in Kentucky that studied the safety and efficacy of inactivated EHV-1 vaccine (Pneumabort K) found that the incidence of EHV-1 abortion was reduced in vaccinated mares compared with unvaccinated control mares [72]. Unfortunately, such studies are problematic from an evidence-based point of view because they are not controlled or masked, and thus do not account for many potential confounding variables. There are certainly no large-scale, double-blind, randomized controlled trials, possibly because of the extraordinary cost of conducting these experiments in horses.

Different studies have found a reduction in the level of viremia in vaccinates with no reduction in the frequency of abortions [73], significantly fewer abortions in vaccinates with no sustained difference in the level or duration of viremia [74], and no evidence of protection against viremia or abortion [68]. The disparity in the data produced by these studies is partially related to the pathogenesis of EHV-1 abortion, because not all infected mares become viremic and not all viremic mares abort. The unpredictable nature of EHV-1 abortion requires that any field trial recruit large numbers of mares. This requirement has not been met by many of the studies done to date. The most significant evidence for the efficacy of an inactivated EHV-1/4 combined vaccine demonstrated that vaccinates were significantly protected against respiratory disease and also had evidence of protection against EHV-1 abortion [74]. This study, however, involved only nine
pregnant mares (five vaccinates and four unvaccinated controls), and although there was a significant difference in the frequency of abortion between vaccinates and controls, one of the vaccinated mares aborted after challenge.

Despite the lack of formal efficacy data from field trials rather than experimental challenge models, inactivated EHV-1/4 vaccines are widely used in the horse industry and are widely associated with a reduction in the frequency of EHV-1 abortion. Data on the frequency of EHV-1 abortion in central Kentucky over the past 5 decades show a reduction in the frequency rate of EHV-1 abortion after the widespread commencement of vaccination with inactivated EHV-1 and, later, EHV-1/4 combined vaccines. It should be remembered, however, that during this same period, our knowledge of the pathogenesis and epidemiology of EHV-1 abortion has increased significantly and farm management practices have been adapted to reduce the impact of EHV-1 abortion storms.

EHV-1 pathogenesis studies have shown that viremia occurs in the presence of high levels of neutralizing antibody [57,58]. Because viremia is central to the pathogenesis of EHV-1 systemic disease, it has been suggested that effective EHV-1 vaccines must stimulate high levels of CTLs [75]. This has led to a recent revival in interest in attenuated EHV-1 vaccines. A temperature-sensitive EHV-1 isolate has been administered by intranasal instillation to pregnant mares, young horses, and foals [76–78]. These studies suggest that attenuated EHV-1 vaccines can be safe and efficacious; however, again, there have been only small numbers of horses tested, and some vaccinated mares aborted after challenge.

EHV-4 infection is ubiquitous in the equine population. Several serosurveys have shown that the level of EHV-4 seropositivity using a type-specific ELISA [52] approaches 100% in mares and foals [60,79,80]. EHV-4 respiratory disease is generally a mild self-limiting condition, but in performance horse stables, any loss of respiratory capacity is important; thus, EHV-4 is potentially an important pathogen. The efficacy of vaccination against EHV-4 respiratory disease has been tested [74]. Weanling foals were initially vaccinated twice, 4 weeks apart, with an EHV-1/4 combined vaccine. Two weeks after the second vaccination, these foals and a group of unvaccinated control foals were infected with EHV-4 by intranasal instillation. The vaccinated group shed less EHV-4 for a shorter duration of time than the control group and exhibited less severe clinical signs of respiratory disease, as determined by the authors’ clinical scoring system.

Summary

There is some evidence that inactivated EHV-1/4 vaccines reduce the likelihood of EHV-1 abortion as well as the severity and duration of EHV-1 or EHV-4 respiratory disease after challenge infection; however, individual vaccinated animals may still experience disease. Recent studies
suggest that some level of protection against EHV-1 abortion is also afforded by intranasal administration of an attenuated EHV-1 isolate. There is no available evidence that vaccination with inactivated or attenuated EHV-1 vaccines can prevent EHV-1 abortion if the challenge dose is high, however, such as might occur when management of the index case of EHV-1 abortion is inappropriate. The available studies on which vaccination recommendations are based all have problems with sample size, experimental design, and analysis, making formation of valid conclusions problematic. None of the published studies outlines the rationale behind the manufacturer’s recommendations to vaccinate pregnant mares in the fifth, seventh, and ninth months of gestation. Importantly, no published studies evaluate the efficacy of EHV-1 vaccination in the prevention of EHV-1 neurologic disease, and no commercially available vaccines make any claim in this regard. There is some evidence to support claims that EHV-4 vaccination reduces the severity of EHV-4 respiratory disease as well as the duration and titer of EHV-4 shed by infected horses.

**West Nile virus**

West Nile fever, caused by infection with West Nile virus (WNV), takes its name from the West Nile district of Uganda, where the virus was first isolated in 1937 from the blood of a febrile woman. The virus, which is transmitted by insect vectors, has a wide geographic distribution, including Africa, the Middle East, Southwest and Central Asia, Europe, and, most recently, North America. For WNV, the principle vectors are various species of mosquito, and the main reservoir hosts are birds. The transmission cycle involves mosquitoes infecting birds and feeding on their blood. The virus is then amplified in the bloodstream of infected birds, which then infect other mosquitoes when they feed. Infected mosquitoes that feed on other animals, including human beings and horses, may infect these hosts, which usually do not become sufficiently infected to allow further transmission. It is believed that migration of infected birds between regions with suitable vectors has resulted in the emergence of the disease in areas, such as the United States, that had not previously seen WNV. Most infected migratory birds and mammals do not usually show clinical signs of infection, although the outbreaks seen in North America have resulted in large numbers of nonmigratory birds and horses demonstrating signs.

An inactivated WNV vaccine for horses has been available in North America since 2002, with a “live canarypox-vectored” vaccine launched commercially in 2004. Among the experimental evidence for WNV vaccination, the plaque reduction neutralization test (PRNT) was used to evaluate the serologic immune responses provided by intramuscular administration of two doses, given 3 weeks apart, of three different potencies of the inactivated vaccine [81]. All three groups demonstrated significant increases in
serum antibody titers when tested 14 days after the second vaccine dose was administered. The medium-dose vaccine group and nonvaccinated controls were subsequently experimentally challenged 12 months after administration of the second vaccine. Nine (82%) of 11 controls compared with only 1 (5%) of 19 vaccinated horses developed viremia after challenge, thereby providing evidence of a statistically significant ($P < .0001$) protective effect from vaccination in this experimental challenge study. Several blind experimental challenge studies have been conducted on the canarypox-vectored vaccine that is licensed in United States to protect against WNV infection (Recombitek WNV). In studies, the canarypox-vectored vaccine induced detectable neutralizing antibodies among all 28 horses administered two doses intramuscularly 5 weeks apart [82]. Using a WNV-infected mosquito feeding challenge, no clinical signs of WNV were detected in any challenged animals. More than 80% of the 16 nonvaccinated controls had detectable viremia compared with none (0%) of 9 horses ($P < .001$) challenged 2 weeks and only 1 (10%) of 10 horses ($P = .001$) infected 12 months after receiving the two-dose primary vaccination. In a parallel study [83], 9 horses that received a single dose of canarypox-vectored vaccine 26 days earlier and 10 nonvaccinated animals were challenged using a WNV-infected mosquito challenge. Viremia was detected in only 1 vaccinated horse compared with 8 nonvaccinated animals ($P = .005$), and although all horses seroconverted after challenge, antibodies were detected sooner among vaccinated horses. The authors suggested that the results indicated some potential benefit from administration of a single dose of this vaccine under some field conditions. Finally, the canarypox-based vaccine was also evaluated using an experimental WNV intrathecal challenge model to assess the protective efficacy of this vaccine when neurologic clinical signs are induced [84]. In this scenario, although the challenge model was artificial, it was believed to resemble the severity of disease encountered in natural disease more closely, which had not been produced in previous mosquito-biting challenge studies. After this challenge, 8 (80%) of 10 controls developed clinical signs of encephalomyelitis, compared with only 1 vaccinated horse (10%) that exhibited a single episode of muscle fasciculation ($P = .005$). Nine controls and 1 vaccinated horse developed fever ($P = .001$). Postmortem histopathologic examination indicated that 8 controls and 1 vaccinated horse had evidence of nonsuppurative encephalitis ($P = .005$). Together, these data provide convincing experimental evidence for the efficacy of inactivated and canarypox-vectored live vaccines against WNV viremia and some evidence for the protective efficacy of live vaccination against the development of clinical signs of disease with WNV infection.

Another study described an experimental WNV-infected mosquito horse-feeding challenge study of a noncommercially available DNA vaccine (plasmid pCBWN) administered intramuscularly on one occasion [85]. In this challenge, all four (100%) vaccinated horses remained healthy and nonviremic, whereas seven (88%) of eight nonvaccinated animals developed
viremia ($P = .01$) and one became febrile and developed neurologic signs 8 days after challenge, requiring euthanasia on day 9 after infection.

Although the experimental vaccine studies described previously provide controlled conditions for assessing the efficacy of vaccination using artificially induced WNV infection and clinical disease, they do not necessarily reproduce the variation in field conditions in which the vaccine is used by practitioners. Therefore, it would be valuable to assess the apparent effectiveness of field WNV vaccination in protecting against clinical disease and mortality. The annual westward spread of WNV across the United States since its first emergence in New York in 1999 and the availability of equine WNV vaccines since 2002 have provided the opportunity for several authors from different states in the United States to assess field vaccine effectiveness.

Vaccine effectiveness among 569 horses naturally exposed to WNV in North Dakota was assessed in 2002 [86]. In a final multivariable logistic regression model ($n = 389$), the odds of death attributable to WNV were significantly reduced (odds ratio [OR] = $0.062$, 95% confidence interval [CI]: 0.007–0.58) among horses that had received vaccine administered according to the manufacturer’s recommendations compared with nonvaccinated animals after controlling for horse’s age and signs of incoordination, caudal paresis, or recumbency. The odds of death were also significantly reduced (OR = 0.32, 95% CI: 0.15–0.68) for horses that received one or two doses of vaccine, even though the dosing was not according to the manufacturer’s recommendations. These data also indicated that a significantly lower proportion of vaccinated horses compared with nonvaccinated animals became recumbent ($P = .018$). In a similar retrospective study conducted among WNV-infected horses in Texas in 2002, investigators showed that after accounting for the clinical signs of ataxia, falling down, recumbency, and lip droop, horses that had been vaccinated at least once before the onset of disease were almost twice as likely to survive (OR = 1.8; $P = .005$) than those that had not been vaccinated in the year before the development of signs [87]. In a prospective cohort study of horses in California between December 2003 and November 2005, the occurrence of clinical disease associated with WNV infection was monitored among 37 nonvaccinated and 155 comingled vaccinated horses (87 receiving inactivated vaccine and 68 receiving canarypox recombinant vaccine). There was serologic evidence for exposure to WNV among 68% of 31 seronegative nonvaccinated horses between December 2004 and the end of the study period, confirming that WNV had been present among the cohort. Two (5%) of the 37 nonvaccinated horses compared with none of the 155 vaccinated animals developed clinical neurologic disease attributable to WNV infection, highlighting a statistically significant protective effect attributable to vaccination ($P = .036$).

Experimental challenge data for the inactivated WNV vaccine were accompanied by field-based safety information from 648 horses, including
32 pregnant mares, that demonstrated absence of local or systemic reactions in 96% of vaccinated animals and mild local reactions in the remainder [81]. Further evidence of safety among 595 pregnant mares was provided in a retrospective study showing there was no increased risk of pregnancy loss among WNV-vaccinated compared with nonvaccinated horses [88]. Under field conditions, the canarypox-vectored vaccine has been shown to provide a good anamnestic serologic response in animals that had previously only been administered the inactivated vaccine [89]. Another field-based study compared the immune responses induced by natural infection in 37 animals with those after vaccination in 187 horses [90]. For animals for which there were data available 5 to 7 months after infection or vaccination, 90% (18 of 20) of naturally infected horses had PRNT titers of 1:100 or greater 5 to 7 months after infection, whereas only 33% (28 of 84) of vaccinated horses had equivalent levels of PRNT titers 5 to 7 months after a second vaccine dose (P < .0001). The authors of this study concluded that revaccination every 6 months in endemic areas, in addition to good preventive methods, may be necessary to prevent WNV in some horses.

Summary

There is a growing body of experimental and field-based evidence that WNV vaccination in horses is effective in preventing viremia and the associated clinical disease and mortality that occur in a small proportion of infected animals. Field data indicate that vaccine manufacturers’ recommendations should be followed but that more regular boosting, especially just before or during extended high-risk periods, may be warranted to maximize protective immunity.

Potomac horse fever (*Neorickettsia risticii*)

Potomac horse fever (PHF) is caused by infection with the small bacterial organism *Neorickettsia risticii*. This organism was formerly called *Ehrlichia risticii*; hence, the disease is frequently also referred to as equine monocytic ehrlichiosis (EME). The disease was first recognized in 1979 in areas along the Potomac River in Maryland and Virginia and has since been recognized more widely in North America and Europe. The disease is seasonal, occurring most commonly in the summer, and is characterized by fever, associated depression and inappetence, diarrhea, and demonstrable leukopenia on whole-blood examination as well as laminitis in approximately 25% of cases. Mortality may be as high as 25%, and abortion can occur in pregnant mares.

Preventive strategies are based on administration of inactivated whole-organism (bacterin) vaccines, all based on a single strain isolated from a horse in Maryland in 1984. In one experimental challenge study in 40
horses, all 13 control horses (100%) developed the disease, whereas only 6 (22%) of 27 vaccinated animals showed clinical signs \( (P < .0001) \) [91]. In contrast, data from a cross-sectional field study of 2587 horses on 511 farms in New York failed to demonstrate a correlation between the county’s seropositive proportion and the percentage of horses vaccinated for PHF [92]. Vaccination was not associated with a reduction in prevalence or severity of clinical signs of EME, and the median date of diagnosis was not delayed compared with nonvaccinated animals [92]. A limited survey of 38 (88%) of 43 PHF cases that occurred between 1994 and 1996 demonstrated a high proportion of vaccine failure [93]. The authors of this survey also demonstrated poor serologic responses among 41 horses that received one \( (n = 5) \), two \( (n = 20) \), or three \( (n = 16) \) doses of vaccine and marked heterogeneity among new \( N. risticii \) isolates. It was thus concluded that the observed failure of vaccination against PHF [92] could be attributable to a combination of deficiency in antibody response by horses receiving even multiple doses and antigenic differences between the existing vaccine strain and new field isolates.

**Summary**

Although the early experimental data, probably based on homologous experimental challenge, provided statistically strong evidence in favor of a clinical benefit from vaccination for PHF, this has not been supported by subsequent data acquired from use of the vaccine in the field. Subsequent investigations have highlighted limited antibody responses and strain differences as possible reasons for poor vaccine effectiveness in the field.

**Equine viral arteritis**

Equine viral arteritis (EVA) is an infectious disease of horses caused by equine arteritis virus (EAV), a member of the family Arteviridae, genus *Arterivirus*. EAV was first isolated from horses during an outbreak of severe respiratory disease and abortion on a Standardbred stud farm in the town of Bucyrus, Ohio in 1953. The consequences of the infection range from subclinical infection to an influenza-like pyretic illness in adult horses, abortion in pregnant mares, and interstitial pneumonia in neonatal foals. Vaccination strategies are based on the use of formalin-inactivated and live-attenuated vaccines, with a geographic split in their use between Europe and Japan (inactivated) and North America (live attenuated). New experimental DNA and protein subunit vaccines are also being developed.

After the outbreak in the United Kingdom in 1993, a formalin-inactivated vaccine (Artervac) has been in use, predominantly in Thoroughbred stallions. Although an archive of data exists to demonstrate that the vaccine is capable of inducing long-lasting neutralizing antibody levels in
animals receiving repeated booster vaccinations, no evidence is available on which to assess its efficacy in preventing establishment of the carrier state in infected stallions.

Another inactivated vaccine against EVA has been developed, and its immunogenicity and efficacy have been studied [94]. Two doses of the vaccine administered a month apart did induce antibodies, but these decreased rapidly. A further dose of vaccine given 2 months after the second dose induced an anamnestic antibody response that persisted for 6 months. Further studies have shown that vaccination with this inactivated vaccine does protect stallions from the carrier state and pregnant mares from abortion [95,96], although it apparently does not protect all animals against EAV infection, because EAV was recovered from the blood of some vaccinated horses after experimental challenge.

Early studies of EAV demonstrated that virulent virus could be attenuated by means of repeated passage through various different cell lines while, at the same time, retaining its ability to stimulate long-lasting immunity (immunogenicity) up to 2 years after vaccination [97,98]. Only the minimal side effects of short-term abnormality of sperm morphology in stallions and mild fever have been reported for the modified live virus (MLV) vaccine [99], although live virus can be recovered transiently from the blood and nasopharynx in some vaccinated animals [100,101]. The MLV vaccine protects against clinical disease and reduces virus shedding, and horses in contact with and mares covered by vaccinated stallions are not infected by EAV [102,103]. The use of MLV vaccine is not recommended in pregnant mares, however, because occasional fetal infections have been described [104].

New vaccines against EAV are under development, including a DNA vaccine that has been demonstrated to induce a long-lasting immune response [105]. Experimental challenge of recombinant subunit EAV vaccines has shown a reduction in the severity of clinical signs and virus shedding [106].

*Summary*

MLV and whole-virus inactivated EVA vaccines are used in geographically distinct regions of the world with little prospect that the alternative type is likely to be adopted outside their now established but limited markets because of different regulatory requirements in different countries; for example, MLV EAV vaccines are not allowed to be used in the United Kingdom. Significant data relating to efficacy exist for the MLV vaccine from experimental and field sources, particularly in preventing the carrier state in vaccinated colts. Concerns remain as to the safety of the MLV vaccine in pregnant mares late in gestation, in inducing vaccine-related clinical disease, and in the possibility of collateral transmission. In contrast, there is less clear-cut evidence to suggest efficacy of the inactivated vaccines. For these vaccines, more frequent boosting and slower onset of protection remain concerns, particularly in high-value stallions beginning their reproductive
Concern also remains for both types of established EVA vaccine regarding their ready differentiation from natural infection. As such, future marker vaccines based on a range of technology, such as subunits, DNA, or viral vectors, might prove persuasive if combined with rapid onset and long-lasting immunity.

Equine encephalitides

There are three antigenically distinct alphaviruses that cause equine encephalitis, also referred to as encephalomyelitis. The three viruses are eastern equine encephalitis (EEE), western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE) viruses, thus called because of their geographic distribution in the Americas, which is where they occur as mosquito vector-borne infections. Live and inactivated virus vaccines have been developed against equine encephalitis virus infection.

The inactivated vaccines are of relatively low immunogenicity and provide only relatively short-lived protection against clinical disease. A recent prospective study showed that horses responded variably to each antigen. Some animals in the study failed to show increased titers despite recent vaccination, and others had low or undetectable antibodies 6 months after vaccination [107]. Subsequent studies have highlighted variation in responses between different commercial vaccines, including EEE antigen, which should be considered in vaccine selection [108].

Inadequate inactivation of the virus possibly caused a major epidemic or epizootic of VEE in Central America and Texas in the 1970s; subsequently, a live-attenuated VEE virus vaccine (TC-83) was developed by cell culture passage. Although inactivated vaccines continue to be used to prevent equine infections with WEE and EEE viruses, live-attenuated vaccines are only available for VEE. An experimental challenge infection of 13 vaccinated and 5 nonvaccinated control horses [109] showed complete protection among the vaccinated horses, but all the controls demonstrated signs of disease ($P < .001$), with 4 control horses dying because of the disease ($P = .002$).

Field studies have also evaluated the safety and efficacy of a live VEE vaccine [110]. In three studies, there was an overall seroconversion rate of 87% (127 of 146 vaccinated horses) observed after vaccination to the epizootic virus strain, although vaccine batch variation was believed to account for the observation of only 50% seroconversion in one population of vaccinated horses studied. Viremia was observed after vaccination in 10 of 26 horses; this was raised as a concern because of the possibility for reversion to virulence of the vaccine strain if viremia levels were high enough to allow infection of mosquitoes. No serious adverse reactions were observed, however, including no vaccine-associated abortion in 42 mares, among 100 horses vaccinated in one of the studies. The authors considered that the cessation of deaths 9 days after vaccination of 900 horses during one
outbreak that had seen approximately 30 VEE-associated deaths around the
time of vaccination and 12 further deaths up to the ninth day after vaccina-
tion was evidence of vaccine efficacy. The lack of nonvaccinated control
animals makes this assertion difficult to corroborate definitively. Another
study, however, demonstrated VEE infection after administration of live-
attenuated vaccine in 10 horses [111].

More recent field observations have demonstrated that widespread vacci-
nation with live-attenuated vaccine, in conjunction with vector control and
movement restrictions, was apparently effective in quickly restricting two
fatal VEE epizootics in Mexico in 1993 and 1996 [112]. The relative effec-
tiveness of the individual measures could not be determined, however, and
some cases did occur among vaccinated horses with apparently adequate
levels of homologous neutralizing antibody. The occurrence of clinical cases
in vaccinated horses highlights potentially significant strain variation be-
tween recent epizootic and conventional live vaccine VEE virus strains. In
addition, although various studies have demonstrated cross-protective im-
munity conveyed from EEE and WEE against experimental and field infec-
tious challenge with VEE [113], there also seems to be interference with VEE
antibody responses with multivalent vaccination [114–118].

The safety, immunogenicity, and efficacy in horses of a new genetically
engineered live-attenuated VEE vaccine candidate, V3526 [119], which had
previously been shown to be the best of 14 potential candidates for a human
vaccine based on animal infection models [120], have also been demon-
strated. Recombinant and DNA vaccines are under investigation in rodent
models and have shown promising results, although trials in horses are
necessary to assess the efficacy of these new vaccines [121].

Summary

VEE is the most studied of the three alphavirus equine encephalitides that
occur in the Western Hemisphere. Experimental and field challenge studies
provide support for the efficacy of live-attenuated VEE virus vaccines. Care
is required in the interpretation of some of this evidence, however, because
of the potential confounding effects of concurrent management practices
during field epizootics and evidence for cross-protective immunity from
vaccinal immunity to EEE and WEE against infection with VEE virus. An-
tigenic differences between vaccine and field virus strains may also be
important.

Strangles (*Streptococcus equi* subsp *equi*)

*Streptococcus equi* subsp *equi* is the causal organism of an acute and
highly contagious upper respiratory disease (strangles) that mainly affects
young horses. Unlike the closely related equine streptococci *S equi* subsp
zooepidemicus, *S equi subsp equi* is not part of the normal bacterial flora of the equine pharynx. These bacteria impinge on the lingual or palatine tonsillar tissue, and hence are translocated to the lymph nodes draining the tonsillar tissue and the pharynx. Typical clinical signs of strangles include nasal discharge, fever, and abscessation of the regional lymph nodes that drain the upper respiratory tract, namely, the retropharyngeal and submandibular lymph chain. Abscessed lymph nodes rupture, if left untreated, to the external environment through the skin or into internal spaces, such as the guttural pouch. Abscess formation can occur at other sites, and this is commonly referred to as “bastard strangles.” Lymph nodes of the head and neck as well as the mediastinal and mesenteric lymph nodes are sites often affected by bastard strangles. Although mortalities from strangles are not common, death can result from rupture of abscesses into the thoracic or abdominal cavity or because of respiratory distress attributable to pressure on the trachea from enlarged abscessed retropharyngeal lymph nodes (hence, the name “strangles”). Purulent discharges from the respiratory tract of affected horses or from draining abscesses are important sources of *S equi subsp equi* from which other horses are infected directly, by horse-to-horse contact, or indirectly by means of shared housing or feeding equipment or by contact with other fomites. In addition to obviously diseased horses, horse-to-horse spread of *S equi subsp equi* can occur from apparently healthy carrier horses in association with guttural pouch infection. Most infected horses recover from the disease and do not become carrier animals [122].

Commercial vaccines have been used to attempt to control strangles for many years in Australia and the United States, with limited evidence of efficacy. Bacterins of inactivated whole *Streptococcus equi subsp equi* or adjuvanted extracts of *S equi subsp equi* have been shown to be immunogenic but have had little published efficacy data to support their use [123]. A study in Australia that surveyed stud farm owners or managers to determine the incidence, risk factors, and effect of vaccination on the occurrence of strangles on horse farms found that studs at high risk from strangles (eg, they had had cases in the recent past) were more likely to vaccinate against strangles but that there was no indication that vaccination decreased the likelihood of outbreaks of strangles [124]. Vaccination with inactivated or extract vaccines has been reported to reduce the severity of clinical signs of strangles [125] and to reduce the attack rates in vaccinated animals after challenge, but adverse reactions to vaccination, such as soreness and abscessation at the injection site, have been reported [123]. The lack of efficacy of these whole-cell or extract vaccines containing high levels of M protein is likely attributable to the type of immune response stimulated after parenteral administration of an inactivated antigen.

Recent research efforts have been directed toward stimulating a local opsonizing mucosal IgA and IgG immune response [126–128]. A live-attenuated intranasal *S equi subsp equi* vaccine has been developed and
commercially produced in the United States [129] in an attempt to stimulate the local mucosal protection observed in recovered animals. Concerns over the balance between safety as a result of sufficient attenuation and protection associated with significant immunogenicity have limited the use of this vaccine outside the United States.

Recently, a genetically attenuated vaccine has been released in the United Kingdom. Lacking the aro A gene required for aromatic amino acid synthesis [130], this deletion mutant has been shown to confer significant protection from challenge after intramucosal administration into the upper lip, but this protection is of short duration, thus requiring three monthly booster vaccinations [131]. Pustules at the site of administration in the lip were a commonly reported adverse reaction to vaccination in this study.

Summary

Although there are several publications that describe the safety and efficacy of a variety of S equi subsp equi vaccines, there is no definitive large-scale field trial to describe the efficacy of vaccination in the “real world.” Although the new-generation vaccines, particularly the intramuscular vaccine, sound promising, further work is required to provide practitioners with the requisite evidence of efficacy in the field setting rather than in the artificial setting of the small-scale challenge trial.

Tetanus

Arguably, the most widely used equine vaccine in the world is the tetanus toxoid, but there are no publications that evaluate the efficacy of this vaccination strategy for horses under extensive field conditions. Tetanus is a sporadic infectious but not contagious disease, and as such, outbreaks of tetanus in horses are not reported. This makes evaluation of vaccine efficacy in the field difficult, because the calculation of attack rates and relative risk of vaccinated versus unvaccinated animals in the face of an outbreak is not possible. All the evidence for the use of this vaccine comes from small-scale antigenicity or challenge trials. Tetanus is an acute toxigenic disease of many species; however, the susceptibility of the different species to tetanus toxin varies considerably. Horses and human beings are highly susceptible, ruminants and pigs are intermediate susceptible, and carnivores are relatively resistant to the effects of tetanus toxin. Tetanospasmin (tetanus toxin) binds to receptors on the neuromuscular junction and is transported to the central nervous system in toxin-containing vesicles by retrograde intra-axonal flow, where it blocks transmission of inhibitory signals, resulting in spastic paralysis [132].

It is certainly true that the absence of evidence is not evidence of absence. Despite the absence of evidence from large-scale trials to demonstrate conclusively that tetanus toxoid effectively prevents tetanus, there is sufficient evidence from small-scale challenge studies and clinical data from
practitioners to support the prophylactic use of tetanus toxoid in horses. A recent case series of horses with tetanus found that the most commonly reported risk factor in the affected horses was the absence of vaccination [133], and this is also consistent with other case reports in which the vaccination history was not known. Challenge trials have demonstrated adequate levels of protection against challenge with purified tetanus toxin [134], and studies have also examined the duration of immunity after multiple doses of toxoid [135,136]. At least two doses of toxoid would seem to be required to ensure that antibody levels were maintained at greater than 0.01 IU, which was the level previously determined to provide sufficient protection [137]. Many horses achieved these levels after a single dose of toxoid, but variation between individual horses was such that a single dose could not be relied on to stimulate adequate levels of antibody to provide protection for a 12-month period [136]. There is little experimental evidence to support the recommended 12-month interval between tetanus booster vaccinations, because all horses that received two tetanus toxoid vaccines 4 weeks apart had high levels of antitetanus antibodies when tested 12 months after the primary vaccine, but the duration of immunity after 12 months was not investigated [136].

It is common practice in equine medicine to use an active-passive immunization approach to protect horses at high risk of developing tetanus. This strategy has been shown to afford rapid onset of protective levels of antibody after the administration of antitoxin, with a long duration of antibody titer, particularly if the dose of toxoid was repeated [135].

Summary

The categoric nature of the evidence of protection against tetanus (immune horses survive, and nonimmune horses die) allows the practitioner to make a valid evidence-based decision to use tetanus toxoid and antitoxin, despite the absence of large-scale double-blind clinical trials. It is the view of the authors that all horses should be vaccinated routinely against tetanus, although the duration of immunity from vaccination has not been established.

References


